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(54) POLYPEPTIDES DE 3-CETOACYLE COA SYNTHASE, ELONGASE D'ACIDES GRAS (54) FATTY ACID ELONGASE 3-KETOACYL COA SYNTHASE POLYPEPTIDES



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Elongase KCS polypeptides with altered substrate specificity and/or catalytic activity are disclosed. Such elongase KCS polypeptides are effective for producing very long chain fatty acids (VLCFA) fatty acids. Also disclosed are nucleic acids encoding elongase KCS polypeptides, and yeast and plants expressing these polypeptides.





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Fatty Acid Elongase 3-Ketoacyl CoA Synthase Polypeptides

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. §119(e) of U.S. provisional application serial number 60/210,326, filed June 8, 2000.

TECHNICAL FIELD

This invention relates to enzymes involved in very long chain fatty acid (VLCFA) synthesis, and more particularly to chimeras and mutants of nucleic acid sequences encoding fatty acid elongase 3-ketoacyl CoA synthase polypeptides.

BACKGROUND

Plant seeds accumulate primarily 16- and 18-carbon fatty acids (FA). Plants also synthesize very long chain fatty acids (VLCFA). VLCFAs are saturated or unsaturated monocarboxylic acids with an unbranched even-numbered carbon chain that is greater than 18 carbons in length. Very long chain fatty acids are key components of many biologically important compounds in animals, plants, and microorganisms. For example, in animals, the VLCFA arachidonic acid is a precursor to many prostaglandins. In plants, VLCFAs are major constituents of triacylglycerols in many seed oils, are essential precursors for cuticular wax production, and are utilized in the synthesis of glycosylceramides, a component of the plasma membrane. Important VLCFAs include arachidic acid (C20·0; i.e., a 20 carbon chain with no double bonds), behenic acid (C22·0), rucic acid (C22·1), and lignoceric acid (C24·1).

VLCFAs are not desirable in edible oils. Oilseeds of the Crucifereae (e.g., rapeseed) and a few other plants, however, accumulate C20 and C22 fatty acids. Although plant breeders have developed rapeseed lines that have low levels of VLCFAs for edible oil purposes, even lower levels would be desirable. On the other hand, vegetable oils having elevated levels of VLCFAs are desirable for certain industrial uses, including uses as lubricants, fuels and as a feedstock for plastics, pharmaceuticals and cosmetics

The biosynthesis in plants of saturated fatty acids up to an 18-carbon chain occurs in the chloroplast. C2 units from acyl thioesters are linked sequentially, beginning with the condensation of acetyl Co-enzyme A (CoA) and malonyl-acyl carrier protein (malonyl-ACP) to form a C4 acyl fatty acid. This condensation reaction is catalyzed by a 3-ketoacyl synthase II (KASII). The enzyme 3-ketoacyl synthase I (KASI) catalyzes the stepwise condensation of a fatty acyl moiety (C4 to C14) with C2 groups and malonyl-ACP to produce a 3-ketoacyl-ACP product that is 2 carbons longer than the original substrate (C6 to C16). The last condensation reaction in the chloroplast, converting C16 to C18, is catalyzed by 3-ketoacyl synthase II (KASII). 3-ketoacyl moieties are also referred to as 8-ketoacyl moieties.

Each elongation cycle involves three additional enzymatic steps in addition to the condensation reaction discussed above. Briefly, the 3-ketoacyl condensation product is reduced to 3-hydroxyacyl-ACP, dehydrated to the encyl-ACP, and reduced to an acyl-ACP. The fully reduced fatty acyl-ACP reaction product then serves as the substrate for the next cycle of elongation.

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The C18:0 saturated fatty acid (stearic acid) can be desaturated to produce a C18:1 fatty acid (oleic acid), which can be transported out of the chloroplast and converted to a C18:2 fatty acid (linoleic acid) or a C18:3 fatty acid (\alpha-linolenic acid). Stearic acid and oleic acid can also be elongated outside the chloroplast to form VLCFAs. The formation of fatty acid slonger than 18 carbons depends on the activity of a fatty acid elongase complex to carry out four reactions similar to those described above for fatty acid synthesis in the chloroplast. The initial reaction is catalyzed by an elongase 3-ketoacyl CoA synthase (elongase KCS) and involves the condensation of a two carbon group from malonyl CoA with a C18:0 or C18:1 fatty acyl CoA substrate. A gene encoding an elongase KCS from Arabidopsis thaliana has been identified and designated FAE1. See, e.g., U.S. Patent No. 6,124,524. The gene product catalyzes the condensation of olocyl CoA and malonyl CoA, leading to the conversion of the C18 substrate to a C20:1 product, eicosenoyl CoA. Mutations have been identified in the A. thaliana FAE1 gene (see WO 96/13582). A. thaliana plants carrying a mutation in FAE1 have significant decreases in the levels of VLCFAs in seeds.

SUMMARY

Despite 85% sequence identity at the amino acid level between the Arabidopsis thaliana FAE1 polypeptide and the Brassica napus polypeptide of GenBank Accession No. AAB72178, the composition of the oil from A. thaliana and B. napus seeds suggests that the enzymes may have different substrate specificities and/or catalytic activity. VLCFAs constitute about 22% of the seed oil of A. thaliana, whereas VLCFAs constitute about 62% of the seed oil in rape. A. thaliana seed oil is primarily eicosenoic acid (about 18%), with a small amount of erucic acid and longer-chain monunusaturated fatty acids (about 2%). In contrast, rapeseed oil has a relatively small amount of cicosenoic acid (about 10%) and relatively larger amounts of crucic acid and longer-chain monunsaturates (about 52%).

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The present invention provides novel polypeptides with altered elongase KCS substrate specificity and/or catalytic activity. One such novel polypeptide comprises three polypeptide segments. The amino-terminal first polypeptide segment has membrane-anchoring properties. It is joined to a second polypeptide segment whose amino acid sequence is residues 75-114 of SEQ ID NO:12 or residues 75-114 of SEQ ID NO:14, followed by a third polypeptide segment having at least 40% sequence identity to the C-terminal 392 amino acids of SEQ ID NO:4. Examples of such polypeptides have the amino acid sequences shown in SEQ ID NOS:12 and 14. The third polypeptide segment can have an aspartic acid residue at the position corresponding to amino acid 307 of SEQ ID NO:4. Examples of such polypeptides have the amino acid sequences shown in SEQ ID NO:20, 22, 34 and 36.

Such polypeptides can catalyze the condensation of a C18 fatty acyl substrate and malonyl CoA, leading to the synthesis of a C20 fatty acyl CoA. The fatty acid substrate can be oleic acid (C18:1), in which case the product formed is eicosenoic acid (C20:1). In some instances, the fatty acid substrate is stearic acid (C18:0) and the product formed therefrom is arachidic acid (C20:0). Such polypeptides often can further catalyze the condensation of malonyl CoA and a C20 fatty acyl substrate, leading to the synthesis of a C22 fatty acyl CoA. The substrate often is eicosenoic acid (C20:1) and the product is erucic acid (C22:1).

The ratio of the C22 fatty acid product to the C20 fatty acid product (C22:1/C20:1) resulting from the activity of such polypeptides can be about 0.20 or greater, about 0.30 or greater, about 0.40 or greater, or about 0.50 or greater as measured in a yeast microsome assay.

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The invention also features a polypeptide comprising in the amino-terminal to carboxy-terminal direction: a first polypeptide segment that has membrane anchoring properties, joined to a second polypeptide segment that has residues 75-114 of SEQ ID NO:2, which is in turn joined to a third polypeptide segment that has at least 90% sequence identity to residues 115-506 of SEQ ID NO:4. An example of such a polypeptide has the amino acid sequence of SEQ ID NO:8. Also featured is a polypeptide comprising in the amino-terminal to carboxy-terminal direction: a first polypeptide segment having at least 80% sequence identity to residues 1-74 of SEQ ID NO:4, joined to a second polypeptide segment having residues 76-114 of SEQ ID NO:4, joined to a second polypeptide segment having at least 40% sequence identity to residues 115-506 of SEQ ID NO:4. An example of such a polypeptide has the amino acid sequence of SEQ ID NO:10. In some embodiments of these polypeptides, the third segment has an aspartic acid at the position corresponding to amino acid 307 of said SEQ ID NO:4. Examples of such polypeptides have the amino acid sequences of SEQ ID NO:46 and SEO ID NO:18.

A plant is also disclosed, comprising at least one exogenous nucleic acid encoding one or more of the novel polypeptides disclosed herein, as well as seeds having such nucleic acids.

Nucleic acid constructs of the invention comprise at least one regulatory element operably linked to the nucleic acid coding sequence for a novel polypeptide. Host cells containing such nucleic acid constructs are disclosed. Such host cells include bacterial cells, fungal cells, insect cells, plant cells and animal cells.

A method of altering very long chain fatty acids in an organism is disclosed. The method comprises introducing an exogenous nucleic acid into the organism. The nucleic acid encodes one or more of the polypeptides described herein. The nucleic acid is expressed in the organism to produce the polypeptide(s), and the very long chain fatty acid content of the organism is increased compared to the very long chain fatty acid content of a corresponding organism that lacks the exogenous nucleic acid or does not

express the exogenous nucleic acid. Suitable organisms include fungi (e.g., yeast), plants, animals, insects and bacteria. Such organisms can produce a higher level of erucic acid than a corresponding organism that lacks or does not express the exogenous nucleic acid.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. For example, the one letter and three letter abbreviations for amino acids and the one-letter abbreviations for nucleotides are commonly understood.

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In addition, the materials, methods and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

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The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the drawings and detailed description, and from the claims.

BRIEF DESCRIPTION OF SEQUENCES

SEQ ID NO:1 is the nucleotide sequence of the *Arabidopsis thaliana FAEl* gene (GenBank Accession No. U29142).

SEQ ID NO:2 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:1 (GenBank Accession No. AAA70154).

SEQ ID NO:3 is the nucleotide sequence of a *Brassica napus* fatty acid elongase
25 KCS (GenBank Accession No. AF009563).

SEQ ID NO:4 is the amino acid sequence of the *B. napus* polypeptide encoded by SEQ ID NO:3 (GenBank Accession No. AAB72178).

SEQ ID NO:5 is the nucleotide sequence of a *B. napus* fatty acid elongase KCS (GenBank Accession No. U50771).

SEQ ID NO:6 is the amino acid sequence of the *B. napus* polypeptide encoded by SEQ ID NO:5 (GenBank Accession No. AAA96054).

SEQ ID NO:7 is a nucleotide sequence encoding a polypeptide designated At114. SEQ ID NO:8 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:7.

- SEQ ID NO:9 is a nucleotide sequence encoding a polypeptide designated At74.
- SEQ ID NO:10 is the amino acid sequence of the polypeptide encoded by SEQ ID

5 NO:9.

NO:11

- SEQ ID NO:11 is a nucleotide sequence encoding a polypeptide designated At114 L91C K92R.
- SEQ ID NO:12 is the amino acid sequence of the polypeptide encoded by SEQ ID
- 10 SEQ ID NO:13 is a nucleotide sequence encoding a polypeptide designated At114 K92R.
 - SEQ ID NO:14 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:13
- SEQ ID NO:15 is a nucleotide sequence encoding a polypeptide designated At114 is G307D.
 - SEQ ID NO:16 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:15.
 - SEQ ID NO:17 is a nucleotide sequence encoding a polypeptide designated At74 G306D.
- 20 SEQ ID NO:18 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:17.
 - SEQ ID NO:19 is a nucleotide sequence encoding a polypeptide designated At114 L91C K92R G307D.
- SEQ ID NO:20 is the amino acid sequence of the polypeptide encoded by SEQ ID 25 NO:19.
 - SEQ ID NO:21 is a nucleotide sequence encoding a polypeptide designated At114 K92R G307D.
 - SEQ ID NO:22 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:21
- 30 SEQ ID NO:23 is a nucleotide sequence encoding a polypeptide designated At254.

- SEQ ID NO:24 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:23.
- SEQ ID NO:25 is a nucleotide sequence encoding a polypeptide designated At173.
- 5 SEQ ID NO:26 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:25
 - SEQ ID NO:27 is a nucleotide sequence encoding a polypeptide designated $$\operatorname{Bn}176$$
 - SEQ ID NO:28 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:27
 - SEQ ID NO:29 is a nucleotide sequence encoding a polypeptide designated At399.

- SEQ ID NO:30 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:29.
- 15 SEQ ID NO:31 is a nucleotide sequence encoding a polypeptide designated Bn399.
 - SEQ ID NO:32 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:31.
- SEQ ID NO:33 is a nucleotide sequence encoding a polypeptide designated Bn 20 G307D.
 - SEQ ID NO:34 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:33.
 - SEQ ID NO:35 is a nucleotide sequence encoding a polypeptide designated At K92R.
- 25 SEQ ID NO:36 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:35.
 - SEQ ID NO:37 is a nucleotide sequence encoding a polypeptide designated At254 G307D.
- SEQ ID NO:38 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:37.

SEQ ID NO:39 is a nucleotide sequence encoding a polypeptide designated At173 G307D.

SEQ ID NO:40 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:39.

5 SEQ ID NO:41 is a nucleotide sequence encoding a polypeptide designated Bn399 G307D.

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SEQ ID NO:42 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:41.

SEQ ID NO:43 is the 3' chimera-specific primer used in the generation of At173.

SEQ ID NO:44 is the 5' chimera-specific primer used in the generation of At173.

SEO ID NO:45 is the 3' chimera-specific primer used in the generation of At114.

SEQ ID NO:46 is the 5' chimera-specific primer used in the generation of At114.

SEO ID NO:47 is the 3' chimera-specific primer used in the generation of At74.

SEO ID NO:48 is the 5' chimera-specific primer used in the generation of At74.

SEQ ID NO:49 is the 3' chimera-specific primer used in the generation of At114 L91C K92R.

SEQ ID NO:50 is the 5' chimera-specific primer used in the generation of At114 L91C K92R.

SEQ ID NO:51 is the 3' chimera-specific primer used in the generation of At114 K92R.

SEQ ID NO:52 is the 5' chimera-specific primer used in the generation of At114 K92R.

SEQ ID NO:53 is the 5' universal primer used in the generation of At-Bn chimeras.

25 SEQ ID NO:54 is the 3' universal primer used in the generation of At-Bn chimeras.

SEQ ID NO:55 is the 5' universal primer used in the generation of Bn-At chimeras.

SEQ ID NO:56 is the 3' universal primer used in the generation of Bn-At
30 chimeras.

DESCRIPTION OF DRAWINGS

Figure 1 shows amino acid sequences of Brassica napus (Bn) elongase KCS polypeptides, Arabidopsis thaliana FAE1 (At) and novel chimeric polypeptides and novel chimeric polypeptides containing site-directed modifications. Sequences corresponding to those derived from At FAE1 are underlined. Site-directed modifications are indicated in bold. One of the Bn elongase KCS sequences shown corresponds to GenBank Accession No. AAB72178; the other B. napus sequence shown corresponds to a second B. napus elongase KCS having GenBank Accession No. AAA96054.

Figure 2 shows nucleotide sequences of Bn elongase KCS, At FAE1 and novel chimeric nucleic acids and novel chimeric nucleic acids containing site-directed modifications. Sequences corresponding to those derived from At FAE1 are underlined. Site-directed modifications are indicated in bold. The two Bn elongase KCS nucleic acid sequences shown encode the two Bn polypeptides shown in Fig. 1. The GenBank

Accession Numbers are AF009563 and U50771, respectively.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

Fatty Acid Elongase KCS Polypeptides

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In one aspect, the invention provides a polypeptide containing the following segments in the amino-terminal to earboxy-terminal direction: a first polypeptide segment having membrane anchoring properties, joined to a second polypeptide segment having the amino acid sequence of residues of 75-114 of SEQ ID NO:12 or SEQ ID NO:14, joined to a third polypeptide segment having at least 40% sequence identity to the C-terminal approximately 392 amino acids of the Brassica napus elongase KCS polypeptide shown in SEQ ID NO:4. For example, polypeptides designated At114 L91C K92R (SEQ ID NO:12) and At114 K92R (SEQ ID NO:14) are provided by the present invention. The primary sequence of the novel polypeptides of the invention are identified by the source and number of amino-terminal residues (e.g., At74 polypeptides have 74 amino-terminal residues from Arabidopsis thaliana), and site-directed modifications are indicated by the

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original amino acid residue, the position of the modification and the new residue (e.g., polypeptides containing a K92R site-directed modification had a K at amino acid position 92 which was modified by site-directed mutagenesis of the nucleic acid to encode an R residue).

The above-described polypeptides include a first polypeptide segment that can serve as a membrane anchor. Such a segment has properties that result in the clongase KCS polypeptide being anchored to a membrane, such as a lipid bilayer, detergent bilayer, micelle, or cell membrane. Possession of membrane anchoring properties may be the result of the primary structure, secondary structure and/or tertiary structure of the segment. For example, the segment may contain one or more transmembrane domain(s). Alternatively, a post-translational modification of an amino acid residue within the segment can result in the polypeptide being anchored to a membrane. Suitable modifications include, but are not limited to, covalent attachment of a lipid (e.g., a glycosyl phosphatidylinositol anchor) or a carbohydrate (e.g., an oligosaccharide). See, Alberts et al., The Cell, 2nd Edition, Garland Publishing, New York, pp 284-298 and Lodish et al., Molecular Cell Biology, 3rd Edition, Scientific American Books, p. 604 and pp. 688-692. The ability of a segment to serve as a membrane anchor can be demonstrated by observing whether a polypeptide having such a segment co-purifies with a membrane fraction. Alternatively, a segment can be a membrane-anchor if, after fusing it to the second and third segments, it is shown that the polypeptide possesses elongase KCS activity in an in vitro yeast microsome assay, since elongase KCS polypeptides are active when anchored to a membrane. As another alternative, computer algorithms, such as Predict Protein or META Predict Protein (see www.embl-

heidelberg.de/predictprotein), can be used to predict the presence of a transmembrane
 domain within a segment, and hence, the ability of that polypeptide segment to serve as a membrane anchor.

Examples of polypeptide segments that can be membrane anchors include, but are not limited to, amino acids 1-74 of A. thaliana FAB1 (SEQ ID NO:2), and amino acid sequences having 40% or greater sequence identity to residues 1-74 of SEQ ID NO:2. For example, amino acids 1-75 of an elongase KCS from B. napus (GenBank Accession No. AAB72178), amino acids 1-75 of B. juncea protein (EMBL Accession No.

CAA71898), amino acids 1-75 of an elongase KCS from $B.\ napus$ (GenBank Accession No. AAA96054), amino acids 29-105 of a putative β -ketoacyl-CoA synthase from $A.\ thaliana$ (GenBank Accession No. AAD22309) and amino acids 8-76 of a fatty acid elongase-like protein from $A.\ thaliana$ (EMBL Accession No. CAB36702) have at least 40% sequence identity to SEQ ID NO:2. In some embodiments, the first polypeptide segment has at least 80% sequence identity, 90% sequence identity, at least 95% sequence identity, or at least 99% sequence identity to amino acids 1-74 of SEQ ID NO:2.

A percent identity for any subject nucleic acid or amino acid sequence (e.g., any of the fatty acid elongase chimeras described herein) relative to another "target" nucleic acid or amino acid sequence can be determined as follows. First, a target nucleic acid or amino acid sequence of the invention can be compared and aligned to a subject nucleic acid or amino acid sequence, preferably using the BLAST 2 Sequences (Bl2seq) program from the stand-alone version of BLASTZ containing BLASTN and BLASTP (e.g., version 2.0.14). The stand-alone version of BLASTZ can be obtained at <www.fr.com> or <www.ncbi.nlm.nih.gov>. Instructions explaining how to use BLASTZ, and specifically the Bl2seq program, can be found in the 'readme' file accompanying BLASTZ. The programs also are described in detail by Karlin et al. (Proc. Natl. Acad. Sci. USA, 87:2264 (1990) and 90:5873 (1993)) and Altschul et al. (Nucl. Acids Res., 25:3389 (1997)).

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Bl2seq performs a comparison between the subject sequence and a target sequence using either the BLASTN (used to compare nucleic acid sequences) or BLASTP (used to compare amino acid sequences) algorithm. Typically, the default parameters of a BLOSUM62 scoring matrix, gap existence cost of 11, a per residue cost of 1 and a lambda ratio of 0.85 are used when performing amino acid sequence alignments. The output file contains aligned regions of homology between the target sequence and the subject sequence. Once aligned, a length is determined by counting the number of consecutive nucleotides or amino acid residues (i.e., excluding gaps) from the target sequence that align with sequence from the subject sequence starting with any matched position and ending with any other matched position. A matched position is any position where an identical nucleotide or amino acid residue is present in both the target and subject sequence. Gaps of one or more residues can be inserted into a target or subject

sequence to maximize sequence alignments between structurally conserved domains (e.g., α -helices, β -sheets, and loops).

The percent identity over a particular length is determined by counting the number of matched positions over that particular length, dividing that number by the length and multiplying the resulting value by 100. For example, if (i) a 1000 nucleotide target sequence is compared to a subject nucleic acid sequence (e.g., SEQ ID NO:21), (ii) the Bl2seq program presents 200 nucleotides from the target sequence aligned with a region of the subject sequence where the first and last nucleotides of that 200 nucleotide region are matches, and (iii) the number of matches over those 200 aligned nucleotides is 180, then the 1000 nucleotide target sequence contains a length of 200 and a percent identity over that length of 90 (i.e., 180 + 200 x 100 = 90).

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It will be appreciated that a nucleic acid or amino acid target sequence that aligns with a subject sequence can result in many different lengths with each length having its own percent identity. It is noted that the percent identity value can be rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 is rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 is rounded up to 78.2. It is also noted that the length value will always be an integer.

Polypeptides of the invention have a second segment which contains amino acid residues, in particular, the amino acid residue corresponding to position 92 in SEQ ID NO:2, that affect elongase KCS substrate specificity. If the residue at position 92 is an arginine residue, the ratio of the C22:1 product to the C20:1 product is higher than the corresponding ratio observed when the residue is a lysine. Accordingly, the second segment (residues 75-114) of Ati14 L91C K92R and Ati14 K92R both possess an R at position 92. Another example of such a polypeptide has the amino acid sequence of SEQ ID NO:2, except that the lysine at amino acid residue 92 is replaced with an arginine. This polypeptide, designated At K92R, has the amino acid sequence shown in SEO ID NO:36.

Some polypeptides of the invention have a third segment that has at least 40% sequence identity to residues 115-506 of SEQ ID NO:4, which are the carboxy-terminal 392 amino acids of the *B. napus* polypeptide. In some embodiments, the third polypeptide segment has at least 50% sequence identity, at least 60% sequence identity, at

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least 70%, 80%, 90%, 95% or 99% sequence identity to the carboxy-terminal 392 amino acids of SEO ID NO:4.

In some embodiments, the third segment has an aspartic acid residue at the position corresponding to amino acid residue 307 of SEQ ID NO:4. An aspartic acid residue at this position is useful for increasing the catalytic activity of an elongase KCS. compared to the catalytic activity of an otherwise similar polypeptide that has a glycine at this position. For example, polypeptides designated At114 G307D, At74 G306D, At114 L91C K92R G307D, At114 K92R G307D, At254 G307D, At173 G307D, Bn G307D and Bn399 G307D have an aspartic acid residue at the position corresponding to residue 307 of SEQ ID NO:4. These polypeptides have SEQ ID NOS: 16, 18, 20, 22, 38, 40, 34 and 42, respectively.

In some embodiments, the third segment contains one or more of the following groups of residues: GNTSSSS at positions corresponding to residues 423-429 of SEQ ID NO:4, HAGG(R/K)A at positions corresponding to residues 391-396 of SEQ ID NO:4, or MGCSAG at positions corresponding to residues 221-226 of SEQ ID NO:4. These groups of residues are among those that are conserved among elongase KCS polypeptides and are thus found in preferred embodiments.

Segments of a polypeptide are joined to one another by covalent bonds, typically peptide bonds. The segments can be joined directly, without any intervening residues between two segments. Alternatively, one segment can be joined indirectly to an adjacent segment by amino acid residues that are situated between the two adjacent segments and are themselves covalently joined to the adjacent segments. In some embodiments, there are one, two or three intervening amino acid residues. In other embodiments, there are four, five, six, seven, eight, nine or ten intervening residues.

A polypeptide of the invention optionally can possess additional amino acid residues at the amino-terminus or the carboxy-terminus. For example, six His-tag or FLAG™ residues may be linked to a polypeptide at the amino-terminus. See, e.g., U.S. Patent Nos. 4,851,341 and 5,001,912. A reporter polypeptide, such as green fluorescent protein, may be fused to the carboxy-terminus. See, for example, U.S. Patent No. 5,491,084.

With respect to polypeptides, "isolated" refers to a polypeptide that constitutes the major component in a mixture of components, e.g., 30% or more, 40% or more, 50% or more, 60% or more, 70% or more, 80% or more, 90% or more, or 95% or more by weight. Isolated polypeptides typically are obtained by purification from an organism that makes the polypeptide, although chemical synthesis is also feasible. As used herein, "enriched" refers to a polypeptide that constitutes 20-30% (by weight) of a mixture of components. Methods of polypeptide purification include, for example, chromatography or immunoaffinity techniques.

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A polypeptide of the invention may be detected by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis followed by Coomassie Blue-staining or Western blot analysis using monoclonal or polyclonal antibodies that have binding affinity for the polypeptide to be detected.

The presence of a polypeptide of the invention may often be detected by measuring elongase KCS activity. An elongase KCS can catalyze the condensation of a C18 fatty acyl substrate and malonyl CoA, leading to the formation of a C20 fatty acyl product. C18 fatty acids include C18:0 (e.g., stearic acid), C18:1 (e.g., oleic acid), C18:2 (e.g., linoleic acid), and C18:3 (e.g., α-linolenic acid). In some embodiments, an elongase KCS can catalyze the conversion of a C20 fatty acyl substrate to a C22 fatty acyl product. An example of a C20:1 fatty acyl substrate is an eicosenoyl substrate. Such a substrate can be converted to a C22:1 fatty acyl product, e.g., an erucyl product.

Some polypeptides may result in an elongase KCS that does not form reaction product(s) at a desired rate. Such clongases and their genes are useful as controls in analyses of product formation by enzymatically active elongase KCS polypeptides. Such inactive elongase KCS polypeptides and their genes can also be useful in studying the regulation (e.g., transcription, translation, and post-translational events) of genes encoding enzymatically active elongase KCS polypeptides. Such elongase KCS polypeptides can be attached to Sepharose beads and used for affinity purification of fatty acyl substrates from crude preparations. In addition, such elongase KCS polypeptides and their genes can also be useful to develop reagents for various purposes, e.g., immunological reagents to monitor expression of a elongase KCS polypeptides or nucleic

acid probes or primers to monitor inheritance of a elongase KCS gene in a plant breeding program.

Products formed in plants by elongase reactions involving an elongase KCS can be subsequently used to form fatty acyl triacylglycerides (TAGs) during seed development. Alternatively, such products can be further elongated to form cuticular lipids, such as waxes.

In yet another aspect, the invention provides a polypeptide containing the following segments in the amino-terminal to carboxy-terminal direction: a first polypeptide segment having at least 80% sequence identity to the first 74 amino acids of the A. thaliana FAEI gene product (SEQ ID NO:2), joined to a second polypeptide segment having amino acids 76-114 of SEQ ID NO:4, joined to a third polypeptide segment having at least 40% sequence identity to the C-terminal 392 amino acids of a B. napus elongase KCS (SEQ ID NO:4). An example of such a polypeptide is At74 (SEQ ID NO:10). This polypeptide possesses an R residue at position 92. Another example is At74 G306D (SEQ ID NO:18), which has a D residue at position 306.

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Another novel polypeptide disclosed herein contains the following segments in the amino-terminal to carboxy-terminal direction: a first polypeptide segment having membrane anchoring properties, joined to a second polypeptide segment corresponding to amino acids 75-114 of SEQ ID NO:2, joined to a third polypeptide segment having at least 90% sequence identity to the C-terminal 392 amino acids of SEQ ID NO:4. An example of such a polypeptide is At114 (SEO ID NO:8).

The invention also features the following polypeptide, comprising in the aminoterminal to carboxy-terminal direction: (a) a first polypeptide segment having at least
90% sequence identity to residues 1-254 of SEQ ID NO:2, joined to (b) a second
25 polypeptide segment having the amino acid sequence of residues 255-506 of SEQ ID
NO:4. An example of such a polypeptide is designated At254 and the amino acid
sequence is shown in Fig. 1 and SEQ ID NO:24.

Another novel polypeptide comprises (a) a first polypeptide segment having at least 85% sequence identity to residues 1-173 of SEQ ID NO:2, joined to (b) a second polypeptide segment having the amino acid sequence of residues 174-506 of SEO ID

NO:4. An example of such a polypeptide is designated At173 and the amino acid sequence is shown in Fig. 1 and SEO ID NO:26.

Another novel polypeptide comprises: (a) a first polypeptide segment having at least 90% sequence identity to residues 1-399 of SEQ ID NO:2, joined to (b) a second polypeptide segment having amino acid residues 400-506 of SEQ ID NO:4. An example of such a polypeptide is designated At399 and the amino acid sequence is shown in Fig. 1 and SEQ ID NO:30. Such a polypeptide can exhibit a product ratio and catalytic activity resembling that of wild-type At FAEI.

The invention also features the following polypeptide, comprising in the aminoterminal to carboxy-terminal direction: (a) a first polypeptide segment having amino acid residues 1-176 of SEQ ID NO:4, joined to (b) a second polypeptide segment having at least 95% sequence identity to residues 177-506 of SEQ ID NO:2. An example of such a polypeptide is designated Bn176 and the amino acid sequence is shown in Fig. 1 and SEQ ID NO:28. In yeast microsome assays, the Bn176 polypeptide exhibits detectable elongase KCS catalytic activity and a C21:1/C20:1 product ratio of about 0.51.

The invention also features the following polypeptide, comprising in the amino-terminal to carboxy-terminal direction: (a) a first polypeptide segment having amino acid residues 1-399 of SEQ ID NO:4, joined to (b) a second polypeptide segment having at least 95% sequence identity to residues 400-506 of SEQ ID NO:2. An example of such a polypeptide is designated Bn399 and the amino acid sequence is shown in Fig. 1 and SEQ ID NO:32. In yeast microsome assays, the Bn399 polypeptide exhibits detectable elongase KCS catalytic activity and a C21:1/C20:1 product ratio of about 0.35.

Elongase KCS Nucleic Acids and Constructs

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The present invention also includes nucleic acids encoding the above-described polypeptides. As used herein, nucleic acid refers to RNA or DNA, including cDNA, synthetic DNA or genomic DNA. The nucleic acids may be single- or double-stranded, and if single-stranded, may be either the coding or non-coding strand. As used herein with respect to nucleic acids, "isolated" refers to (i) a naturally-occurring nucleic acid encoding part or all of a polypeptide of the invention, but free of sequences, i.e., coding sequences, that normally flank one or both sides of the nucleic acid encoding polypeptide

in a genome; (ii) a nucleic acid incorporated into a vector or into the genomic DNA of an organism such that the resulting molecule is not identical to any naturally-occurring vector or genomic DNA; or (iii) a cDNA, a genomic nucleic acid fragment, a fragment produced by polymerase chain reaction (PCR) or a restriction fragment. Specifically excluded from this definition are nucleic acids present in mixtures of nucleic acid molecules or cells.

Examples of such nucleic acids include those encoding polypeptides designated At114, At74, At114 L91C K92R, At114 K92R, At114 G307D, At74 G306D, At114 L91C K92R G307D, At114 K92R G307D, At154 K92R G307D, At114 K92R G307D, At154, At173, Bn176, At399, Bn399 and At K92R. These nucleic acids have SEQ ID NOS: 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31 and 35, respectively. It should be appreciated that nucleic acids having a nucleotide sequence other than the specific nucleotide sequences disclosed can still encode a polypeptide having the exemplified amino acid sequence. The degeneracy of the genetic code is well known to the att; i.e., for many amino acids, there is more than one nucleotide triplet that serves as the codon for the amino acid.

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Further provided are nucleic acid constructs comprising the above-described nucleic acid coding sequences. Such constructs may be incorporated into a cloning vector. Cloning vectors suitable for use in the present invention are commercially available and used routinely by those of ordinary skill. Nucleic acid constructs of the invention may additionally comprise one or more regulatory elements operably linked to a nucleic acid coding sequence. Such regulatory elements may include promoter sequences, enhancer sequences, response elements or inducible elements that modulate expression of a nucleic acid sequence. As used herein, "operably linked" refers to positioning of a regulatory element in a construct relative to a nucleic acid coding sequence in such a way as to permit or facilitate expression of the encoded polypeptide. The choice of element(s) that may be included depends upon several factors, including, but not limited to, replication efficiency, selectability, inducibility, targeting, the level of expression desired, ease of recovery and the ability of the host to perform post-translational modifications.

The term "host" or "host cell" includes not only prokaryotes, such as E. coli, but also eukaryotes, such as fungal, insect, plant and animal cells. Animal cells include, for

example, COS cells and HeLa cells. Fungal cells include yeast cells, such as
Saccharomyces cereviseae cells. A host cell can be transformed or transfected with a
DNA molecule (e.g., a vector) using techniques known to those of ordinary skill in this
art, such as calcium phosphate or lithium acetate precipitation, electroporation,
lipofection and particle bombardment. Host cells containing a vector of the present
invention may be used for such purposes as propagating the vector, producing a nucleic
acid (e.g., DNA, RNA, antisense RNA) or expressing a polypeptide or fragments thereof.
A nucleic acid encoding a novel polypeptide of the invention may be obtained

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using standard molecular biology techniques, for example, molecular cloning, DNA synthesis, and the polymerase chain reaction (PCR). PCR refers to a procedure or technique in which target nucleic acids are amplified. PCR can be used to amplify specific sequences from DNA as well as RNA, including sequences from total genomic DNA or total cellular RNA. Various PCR methods are described, for example, in PCR Primer: A Laboratory Manual, Dieffenbach, C. & Dveksler, G., Eds., Cold Spring Harbor Laboratory Press, 1995. Generally, sequence information from the ends of the region of interest or beyond is employed to design oligonucleotide primers that are identical or similar in sequence to opposite strands of the template to be amplified. Various PCR strategies are available by which site-specific nucleotide sequence modifications can be introduced into a template nucleic acid.

Nucleic acids of the present invention may be detected by methods such as ethidium bromide staining of agarose gels, Southern or Northern blot hybridization, PCR or in situ hybridizations. Hybridization typically involves Southern or Northern blotting (see, for example, sections 9.37-9.52 of Sambrook et al., 1989, "Molecular Cloning, A Laboratory Manual", 2nd Edition, Cold Spring Harbor Press, Plainview; NY). Probes should hybridize under high stringency conditions to a nucleic acid or the complement thereof. High stringency conditions can include the use of low ionic strength and high temperature washes, for example 0.015 M NaCl/0.0015 M sodium citrate (0.1X SSC), 0.1% sodium dodecyl sulfate (SDS) at 65°C. In addition, denaturing agents, such as formamide, can be employed during high stringency hybridization, e.g., 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C.

Transgenic Plants

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The invention provides a plant containing an exogenous nucleic acid that encodes a polypeptide of the invention, e.g., nucleic acids encoding a polypeptide having an amino acid sequence as shown in SEQ ID NOS:8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36.

Accordingly, a method according to the invention comprises introducing a nucleic acid construct into a plant cell and producing a plant (and progeny of such a plant) from the transformed cell. Techniques for introducing exogenous nucleic acids into monocotyledonous and dicotyledonous plants are known in the art, and include, without limitation, Agrobacterium-mediated transformation, viral vector-mediated transformation, electroporation and particle gun transformation, e.g., U.S. Patents 5,204,253 and 6,013,863. If cell or tissue cultures are used as the recipient tissue for transformation. plants can be regenerated from transformed cultures by techniques known to those skilled in the art. Transgenic plants may be entered into a breeding program, e.g., to introduce a nucleic acid encoding a polypeptide into other lines, to transfer the nucleic acid to other species or for further selection of other desirable traits. Alternatively, transgenic plants may be propagated vegetatively for those species amenable to such techniques. Progeny includes descendants of a particular plant or plant line. Progeny of an instant plant include seeds formed on F1, F2, F3, and subsequent generation plants, or seeds formed on BC1, BC2, BC3, and subsequent generation plants. Seeds produced by a transgenic plant can be grown and then selfed (or outcrossed and selfed) to obtain seeds homozygous for the nucleic acid encoding a novel polypeptide.

In another aspect, the invention provides a method of altering very long chain fatty acids in an organism. The method involves introducing an exogenous nucleic acid into the organism. The organism may be, for example, a yeast or a plant. A nucleic acid construct of the invention can alter the levels of very long chain fatty acids in plant tissues expressing the novel polypeptide, compared to VLCFA levels in corresponding tissues from a plant that does not contain or does not express the polypeptide. A comparison can be made, for example, between a transgenic plant of a plant line and a plant of the same line that lacks the nucleic acid construct or does not express the nucleic acid construct in

that tissue. Plants having an altered VLCFA composition may be identified by techniques known to the skilled artisan, e.g., thin layer chromatographic or gas-liquid chromatographic (GLC) analysis of the appropriate plant tissue. Novel polypeptides can catalyze the conversion of oleic acid (18:1) to eicosenoic acid (20:1), and the conversion of eicosenoic acid to erucic acid (22:1). In some embodiments, the ratio of erucic acid to eicosenoic acid (22:1/20:1) is greater than or equal to 0.20, as measured in the yeast microsome assay described below.

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A suitable group of plants with which to practice the invention include dicots, such as alfalfa, soybean, rapeseed (high crucic and canola), safflower, or sunflower, and monocots, such as corn, wheat, rye, barley, rice, or sorghum. Suitable rapeseed species include B. napus, B. rapa, B. juncea, and B. hirta. Additional plant species suitable for use in the present invention include Sinapsis alba, Crambe abyssinica, Limnanthes douglasti and L. alba.

Suitable tissues in which to express polynucleotides and/or polypeptides of the invention include, without limitation, seeds, stems and leaves. Seeds expressing a novel coding sequence can be used to extract an oil having elevated levels of eicosenoic acid and/or crucic acid. Leaf tissues in which a novel coding sequence can be expressed include cells and tissues of the epidermis, e.g., cells that are involved in forming trichomes. Also of interest are epidermal cells involved in forming the cuticular layer. The cuticular layer comprises various very long chain fatty acids and VLCFA derivatives such as alkanes, esters, alcohols and aldehydes. Increasing the amount of VLCFAs in epidermal cells and tissues may enhance defense mechanisms and drought tolerance of plants.

The invention will be further described in the following examples, which do not
25 limit the scope of the invention described in the claims.

EXAMPLES

Example 1-Construction and Cloning of Nucleic Acids

Nucleic acids encoding chimeric polypeptides were generated by an overlap polymerase chain reaction (PCR) strategy. Horton et al. (1989), Gene, 77:61-68 and see Figure 1 of Ho et al. (1989), Gene, 77:51-59. Briefly, a first round of PCR products were

generated in separate reactions using Arabidopsis thaliana FAE1 and Brassica napus elongase KCS nucleic acid as template. Nucleic acid sequences of the A. thaliana FAE1 and B. napus elongase KCS templates are shown in SEO ID NO:1 and 3, respectively. The portion of each template that was amplified corresponded to the segment to be combined in a desired chimera. The amino-terminal fragment of a given chimera was amplified using a 5' universal primer (sense) and a 3' chimera-specific primer (antisense). The carboxy-terminal fragment of a given chimera was amplified with a 5' chimera-specific primer (sense) and a 3' universal primer (anti-sense). Universal primer sequences are shown in Table 1 and SED ID NOS: 53-56. Chimera-specific primer sequences are shown in Table 2 and SEQ ID NOS:43-52. The 5' and 3' universal primers anneal to the 5' and 3' ends of the template nucleic acid, respectively, and contain BamHI and EcoRI restriction sites, respectively, for ease in subcloning into an expression vector. The 5' chimera-specific primers are antisense to the amino-terminal template and the 3' chimera-specific primers are antisense to the carboxy-terminal template. The 5' and 3' chimera-specific primers each contain an internal complementary sequence where a switch occurs from the At to Bn sequence, or alternatively, from Bn to At.

The products produced by the first round of PCR were purified, and a second round of PCR was conducted using a mixture of the products from the first round of PCR as template nucleic acid. The appropriate 5' and 3' universal primers were used to generate the chimeric nucleic acid product in the second round PCR. The amplified product was then digested with BamHI and EcoRI, ligated into pYES2 (Invitrogen, Carlsbad, CA) and transformed into E. coli. pYES2 is a yeast centromere-containing, episomal plasmid that is stably propagated in both E. coli and in yeast. Each nucleic acid was inserted downstream of the GAL1 promoter in pYES2. The GAL1 promoter is induced in yeast when galactose is present in the medium and repressed when glucose is present in the growth medium.

Nucleic acids encoding polypeptides with site-directed alterations in the coding sequence were also prepared by overlap PCR, using 5' and 3' chimera-specific primers in which the internal complementary region contained the desired sequence modification.

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TABLE 1

Chim	era type		
5' portio	3' portion	5' universal primer	3° universal primer
At	Bn	5'-ggggatccatgacgtccgttaacgttaagctcc-3' (SEQ ID NO:53)	5'-ccgaattettaggaccgaccgttttggacac-3' (SEQ ID NO:54)
Bn	At	5'-ggggatccatgacgtccattaacgtaaagctcc-3' (SEQ ID NO:55)	5'-ccgaattcffaggaccgaccgttttggacatgagtctt-3' (SEQ ID NO:56)

TABLE 2

Chimera	3' chimera-specific primers	5' chimera-specific primers
At173	5'-gegetegaaaatetatteaagaaca-3' (SEQ ID NO:43)	5'-gttettgaatagatttttegagegeaeegatgat-3' (SEQ ID NO:44)
At114	5'-cggaacggcacgtgtgatgattcgtcct-3' (SEQ ID NO:45)	5'-aggacggatcatcacacgcgacgttccg-3' (SEQ ID NO:46)
At74	5'-cccaaaccggtttacctcgttga-3' (SEQ ID NO:47)	5'-tvaacgaggtaaaccggattggg-3' (SEQ ID NO:48)
At114 L91C K92R	5'-ccgcattgcagagttagtgtctctaaa-3' (SEQ ID NO:49)	5'-tttagagacactaactetgeaatgegg-3' (SEQ ID NO:50)
At114 K92R	5'-ccaccgcatctcagagttagtgtctct-3' (SEQ ID NO:51)	5'-agagacactaactctgagatgcggtgg-3' (SEQ ID NO:52)

5 Due to a degeneracy in the primer used to generate the nucleic acids encoding carboxy-terminal sequences from B. napus, the amino acid residue at the fifth to last position from the carboxy-terminus in the polypeptides designated At114, At114 L91C K92R, At114 K92R and At254 is a P and the polypeptide designated At74 is a O at that position as indicated in Fig. 1. The polypeptides designated At 173 and At 399 may have a P or a Q at this position and are shown as Q in Fig. 1. A Q is found in the wild-type Bn 10 polypeptide sequence at this position. In addition, due to PCR infidelity in the preparation of the nucleic acid encoding At114, the amino acid residue at position 439 of SEQ ID NO:8 may be an A or a T, with an A being found in the wild-type Bn sequence. In addition, PCR infidelity in the preparation of the nucleic acid encoding At114 L91C K92R resulted in the residue at position 119 being an N. Position 119 in the wild-type Bn 15 amino acid sequence is a D. Based on the data presented below, this residue can be either a D or an N without any apparent effect on activity.

Mutagenesis was confirmed by automated DNA sequencing, and each construct was used to transform S. cerevisiae strain InvScl (Invitrogen) using a lithium-acetate procedure (Gietz, R. and Woods, R., in Molecular Genetics of Yeast: Practical Approaches, Oxford Press, pp. 121-134 (1994)).

Example 2-Fatty Acid Elongase KCS Activity in Yeast Microsomes

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Elongase KCS enzymatic activity was analyzed by preparing microsomes from transformed yeast cells and assaying these microsomes in vitro for elongase KCS activity. Transformed yeast cells were grown overnight in YPD media at 30°C with vigorous shaking. Complete minimal uracil dropout media (cm-ura) supplemented with galactose (2% weight/volume in 40 ml) was inoculated to an OD600 of 0.002 to 0.01. Cultures were grown at 30°C to an OD600 of approximately 1.5 to 2.0. Cells were harvested by centrifugation at 5000 xg for 10 min and washed with 10 ml ice cold isolation buffer (IB), which contains 80 mM Hepes-KOH (pH 7.2), 5 mM EGTA, 5 mM EDTA, 10 mM KCl, 320 mM sucrose and 2 mM DTT). Cells were then resuspended in enough IB to fill a 1.7 ml tube containing 700 µl of 0.5 µm glass beads and yeast microsomes were isolated from the cells essentially as described in Tillman, T. & Bell, R., J. Biol. Chem. 261:9144-9149 (1986). The microsomal membrane pellet was recovered by centrifugation at 252,000 xg for 60 min. Microsomal pellets were resuspended in a minimal volume of IB, and the protein concentration adjusted to 2.5 µg µI⁻¹ by addition of IB containing 15% glycerol. Microsomes were frozen on dry ice and stored at -80°C. The protein concentration in microsomes was determined by the Bradford method (Bradford, Anal. Biochem., 72:248-54, 1976).

Elongase KCS activity was measured essentially as described in Hlousek-Radojcic, et al., *Plant J.* 8:803-809 (1995). Briefly, the standard elongation reaction mix contained 80 mM Hepes-KOH (pH 7.2), 20 mM MgCl₂, 500 μM NADPH, 100 μM malonyl-CoA, 10 μM CoA-SH and 15 μM [¹⁴C]18:1-CoA (50 μCi μmol⁻¹). The reaction was initiated by the addition of yeast microsomes (6 μg protein) and the mixture was incubated at 30°C, in a final reaction volume of 25 μl. Reaction time was 10 min unless indicated otherwise.

Methyl esters of the acyl-CoA elongase products were prepared by incubation with 500 µl 2% H₂SO₄/MeOH at 80°C for 2 h. Extracted methyl esters were separated on reverse phase silica gel TLC plates (Analtech, Newark, DE), quantified by phosphorimaging, and analyzed by ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA). The detection limit for each product is about 0.001 nmoles/min/mg microsomal protein, depending on the phosphorimage exposure time.

Example 3 - Elongase KCS Substrate Specificity

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Table 3 is a summary of elongase activity and product ratios of *B. napus* (Bn) and

A. thaliana (At) elongase KCS nucleic acid sequences expressed in yeast and assayed as
described in Example 2. Microsomes prepared from galactose-induced yeast expressing
the indicated nucleic acid were assayed after 10 min for conversion of labeled oleoyl
substrate to eicosenoyl product, erucyl product, and lignoceryl product. For convenience,
fatty acyl substrates and products are oftentimes referred to as the acid rather than as the
acyl or acyl CoA. The ratio of 22:1 product to 20:1 product is also shown. Experiments
were performed on 17 individual yeast transformants for each construct.

TABLE 31

		18:1(±sd)	20:1(±sd)	22:1(±sd)	20:1+22:1(±sd)	22:1/20:1(±sd)
i	B. napus elongase KCS (SEQ ID NO:4)	45±4	3.3±0.4	1.4±0.5	4.8±0.2	0.43±0.11
	A. thaliana FAE1 (SEQ ID NO:2)	29±9	6±0.8	1.2±0.2	7.1±0.9	0.20±0.04

Amounts of oleic acid (18:1), eicosenoic acid (20:1), erucic acid (22:1), and the sum of 20:1 and 22:1, are expressed as nmol/mg microsomal protein; ±sd = standard deviation.

Table 4 shows the ratio of 22:1/20:1 products produced by Bn, At, and various chimeric polypeptides after incubation of the microsomes with the labeled 18:1 substrate for 5, 10 or 20 min. The results shown in Table 4 represent 4 different microsome preparations from a single yeast transformant with each construct and 2-3 assays of each microsomal preparation. The At FAEI (SEQ ID NO:2) produces about 5 times more eicosenoic acid than erucic acid. In contrast, the Bn elongase KCS (SEQ ID NO:4) produces about 2-3 times more eicosenoic acid than erucic acid. See also Table 3.

The At254, At173 and At114 polypeptides have a 22:1/20:1 product ratio that is similar to that of wild-type At FAE1, whereas the At74 polypeptide has a product ratio that is similar to that of wild-type Bn (Table 4). These results indicate that amino acids affecting product specificity are present between residues 75 and 114 of the wild-type At elongase KCS. The At74 gene product possesses the amino acid sequence of the Bn elongase KCS of SEQ ID NO:4 at positions 75 to 114, indicating that amino acids of the Bn elongase KCS that differ from the at FAE1 in this region contribute to the difference in C22:1/C20:1 product ratio.

TABLE 4

Time (min)			Pol	ypeptide Assa	peptide Assayed ¹						
	Bn (SEQ ID NO:4)	At (SEQ ID NO:2)	At254 (SEQ ID NO:24)	At173 (SEQ ID NO:26)	Atl 14 (SEQ ID NO:8)	At74 (SEQ ID NO:10)	At114 L91C K92R (SEQ ID NO:12)				
5	0.35±0.07	0.18±0.04	0.14±0.03	0.11±0.07	0.17±0.04	0.42±0.07	0.22±0.02				
10	0.33±0.10	0.13±0.01	0.11±0.03	0.08±0.01	0.15±0.03	0.36±0.06	0.20±0.02				
20	0.35±0.13	0.13±0.02	0.12±0.03	0.11±0.05	0.16±0.04	0.29±0.05	0.20±0.04				

The data are the C22:1/C20:1 ratio ± standard deviation

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Site-directed modifications were made to the At114 or At74 nucleic acid sequence within the region corresponding to residues 75 to 114 in order to determine which amino acids contributed to the altered product ratio. The modified nucleic acids were made according to the overlap PCR strategy described in Example 1 and the constructs were introduced into yeast. Elongase KCS activity was measured as described in Example 2. The results showed that changing the At114 amino acid sequence from alanine to serine and glutamine to lysine at positions 157 and 163, respectively, resulted in undetectable clongase activity. Likewise, changing serine and isoleucine at positions 93 and 95 within At74 to valine in both positions also resulted in undetectable elongase activity.

However, when the leacine and lysine residues at positions 91 and 92 within the At114 polypeptide were changed to cysteine and arginine, respectively, the C22:1/C20:1 product ratio of the resulting polypeptide, At114 L91C K92R, was shifted to more closely resemble that of the wild-type Bn polypeptide (Table 4).

Site-directed modifications were made to the Atl14 nucleic acid sequence to generate coding sequences for two new polypeptides, one bearing the leucine to cysteine modification and one bearing the lysine to arginine modification. These polypeptides were designated Atl14 L91C and Atl14 K92R. The nucleotide sequence of the nucleic acid encoding Atl14 K92R is shown in SEQ ID NO:13 and the amino acid sequence of the polypeptide is shown in SEQ ID NO:14. The two nucleic acids were introduced into yeast and the activity of each polypeptide was analyzed in yeast microsome assays. The results showed that the L to C-modified polypeptide, Atl14 L91C, had low but detectable catalytic activity. The K to R-modified polypeptide, Atl14 K92R, had a higher 22:1/20:1 ratio that approached that of wild-type Bn (Table 5). Results presented are the mean of 1 to 3 individual assays each of at least 7 separate microsomal preparations.

TABLE 5

	20:1+22:11	(22:1/20:1)
At (SEQ ID NO:2)	16.0 +/- 2.7	0.15 +/- 0.04
Bn (SEQ ID NO:4)	9.8 +/- 3.2	0.32 +/- 0.07
At114 K92R (SEQ ID NO:14)	5.8 +/- 3.1	0.32 +/- 0.09

¹⁵ The sum of the amounts of eicosenoic acid (20:1) and erucic acid (22:1) is expressed as nmole/mg microsomal protein.

Example 4-Elongase KCS Catalytic Activity

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Table 6 shows the results of yeast microsome assays of Bn elongase KCS, At FAE1, and various chimeric polypeptides for various incubation times. The data in Table 6 show the sum of C20:1 and C22:1 in nmole/mg protein from microsome preparations assayed 2 to 3 times each.

The results indicate that the amount of elongase KCS activity of the wild-type At FAE1 is about 1.5 to 2 times higher than that of wild-type Bn elongase KCS. The At114 polypeptide has an activity that is intermediate between the wild-type At and wild-type Bn, while the At74 polypeptide has an activity that is lower than that of wild-type Bn enzymes. These results indicate that modifying amino acid residues in the region from position 74 to 114 affects elongase activity.

The activity of the At114 L91C K92R gene product was measured in yeast microsomes and is shown in Table 6. The elongase activity of this polypeptide was higher than that of At114.

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TABLE 61

Time (min)	pYES2	Bn (SEQ ID NO:2)	At (SEQ ID NO:4)	At114 (SEQ ID NO:8)	At74 (SEQ ID NO:10)	At114 L91C K92R (SEQ ID NO:12)
0	-2.4±2.4	2.7±1.8	2.4±1.8	1.4±1.0	2.2±0.84	2.3±1.08
5	0.24±0.6	5.1±1.6	8.7±1.2	5.9±0.6	3.2±1.1	6.5±1.0
10	0.78±0.6	7.4±1.8	12.1±0.6	8.8±0.5	4.1±0.8	9.6±1.2
20	0.96±0.6	7.8±2.1	13.7±1.8	10.1±1.2	4.4±0.8	11.5±1.1
45	1.32±0.6	8.1±2.2	14.0±0.6	10.2±1.2	4.6±0.5	12.1±0.9

¹ The data are the sum of the C20:1 and C22:1 elongase products (nmole/mg microsomal protein) ± standard deviation.

The clongase activity of the At114 L91C and At114 K92R polypeptides were also assayed in yeast microsomes. The results indicated that the catalytic activity of the At114 L91C polypeptide was about 15-30% of the activity of At114, whereas the activity of At114 K92R was approximately the same as that of At114.

A yeast microsome assay was carried out to compare the Bn elongase KCS shown in SEQ ID NO:4 and another naturally-occurring elongase KCS from the *B. napus* cultivar Askari. The elongase KCS from Askari has the same sequence as that shown in SEQ ID NO:4, except for a valine at position 4 and an aspartic acid at position 307. The results indicated that the Askari elongase KCS had a higher elongase activity and a higher C22:1/C20:1 ratio that did the Bn elongase KCS of SEO ID NO:4.

Site-directed modifications to SEQ ID NO:3 were made by the techniques described in Example 2 to generate nucleic acids encoding polypeptides Bn I4V, Bn G307D and Bn I4V G307D. The latter polypeptide has the same amino acid sequence as the naturally occurring Askari elongase KCS. After cloning and transforming of each construct into yeast as described in Example 2, microsome assays were performed. Table 7 presents the results from a single experiment in which elongase activity and product ratios for the elongase KCS constructs were measured. Assays were performed as

described in Example 2. The results indicate that changing the residue at position 4 from isoleucine to valine had little or no effect on the elongase activity or the C22:1/C20:1 ratio. On the other hand, the Bn G307D polypeptide had a higher elongase activity and produced more C22:1 product than did the unmodified wild-type Bn polypeptide. The amino acid sequence of Bn G307D is shown in SEO ID NO:34.

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10

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TABLE 71

	18:1	20:1	22:1	24:1	22:1/20:1	20:1+22:1
Bn	47.9	5.5	1.9	0.3	0.35	7.7
Bn I4V	48.4	5.5	2.0	0.4	0.37	7.8
Bn G307D	37.2	6.7	5.4	0.7	0.80	12.7
Bn I4V G307D	41.9	6.5	4.5	0.5	0.68	11.6
B. napus (Ask)	37.6	7.7	6.7	0.8	0.86	15.2

Amounts of oleic acid (18:1), eicosenoic acid (20:1), erucic acid (22:1), lianoceric acid (24:1), and the sum of 20:1 and 22:1, are expressed as nmol/mg microsomal protein.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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205

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His	Cys	Ile	Asp	Arg 485	tac Tyr	Pro	Val	Lys	Ile 490	Asp						1488
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<213> Artificial Sequence

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<223> 5' 114 amino acids from A. thaliana FAE1 (SEQ ID NO:2) and 3' 392 amino acids from B. napus elongase KCS (SEQ ID NO:4); designated At114

-400-

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330

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Phe	Lys 370	Asp	Lys	Ile	Lys	His 375	тух	тут	Val	Pro	Asp 380	Phe	Lys	Leu	Ala	
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WO 01/94565 PCT/US01/18737

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got tot egg oft acc ata aac gat oft cac aac the oft too tat oft Ala Ser Arg Leu Thr Ile Asn Asp Leu His Asn Phe Leu Ser Tyr Leu 35 40 45	144
cas cac as ctt ats acs gts act tts ctc ttt gct ttc act gtt ttc oln mis Asn Leu Tle Thr Val Thr Leu Leu Phe Ala Phe Thr Val Phe 50 $$	192
ggt ttg gtt ete tac ate gta ace ega ece aat eeg gtt tat ete gtt Gly Leu Val Leu Tyr Ile Val Thr Arg Pro Asn Pro Val Tyr Leu Val $_{65}$ $_{70}$ $_{80}$	240
gac tac tog tgt tac oft cog coa cog cai tgc aga gtt agt gtc tot hasp Tyr Ser Cys Tyr Leu Pro Pro Pro His Cys Arg Val Ser Val Ser 85 90 95 95 85 90 95 85 97 95 88 97 95 98 98 99 98 99 99 99 99 99 99 99 99 99	288
aaa gtc atg gat att ttc tac caa ata aga aaa gct gat act tct tca Lys Val Met Asp Ile Phe Tyr Gln Ile Arg Lys Ala Asp Thr Ser Ser 100 105 110	336
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acg gag caa gtt atc att ggt gcg cta gaa aat cta ttc aag aac acc Thr Glu Gln Val Ile Ile Gly Ala Leu Glu λ sn Leu Phe Lys λ sn Thr 165 170 175	528
aac gtt aac cct aaa gat ata ggt ata ctt gtg gtg aac toa agc atg Asn Val Asn Pro Lys Asp Ile Gly Ile Leu Val Val Asn Ser Ser Met 180 185 190 1	576

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Glu	Leu	Ala 435	Tyr	Ile	Glu	Ala	Lys 440	Gly	Arg	Met	Lys	Lys 445	Gly	Asn	Lys	
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						aac Asn				taa						1521
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Ala	Ser	Arg 35	Leu	Thr	Ile	Asn	Asp 40	Leu	His	Asn	Phe	Leu 45	Ser	Tyr	Leu	
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	aac Asn								576
	cca Pro 195								624
	agc Ser								672
	gtt Val								720
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<223> 5' 114 amino acids from A. thaliana FAE1 (SEQ ID NO:2) and 3' 392 amino acids from B. napus elongase KCS (SEQ ID NO:4), having a mutation at position 92; designated At114 K92R

<400> 14 Met Thr Ser Val Asn Val Lys Leu Leu Tyr Arg Tyr Val Leu Thr Asn 10 Phe Phe Asn Leu Cys Leu Phe Pro Leu Thr Ala Phe Leu Ala Gly Lys 25 Ala Ser Arg Leu Thr Ile Asn Asp Leu His Asn Phe Leu Ser Tyr Leu 40 Gln His Asn Leu Ile Thr Val Thr Leu Leu Phe Ala Phe Thr Val Phe 55 Gly Leu Val Leu Tyr Ile Val Thr Arg Pro Asn Pro Val Tyr Leu Val Asp Tyr Ser Cys Tyr Leu Pro Pro Pro His Leu Arg Val Ser Val Ser 90 Lys Val Met Asp Ile Phe Tyr Gln Ile Arg Lys Ala Asp Thr Ser Ser 100 105 Arg Asn Gly Thr Cys Asp Asp Ser Ser Trp Leu Asp Phe Leu Arg Lys 115 120 125 Ile Gln Glu Arg Ser Gly Leu Gly Asp Glu Thr His Gly Pro Glu Gly 135 140 Leu Leu Gln Val Pro Pro Arg Lys Thr Phe Ala Ala Ala Arg Glu Glu 150 155 Thr Glu Gln Val Ile Ile Gly Ala Leu Glu Asn Leu Phe Lys Asn Thr 165 1.70 175 Asn Val Asn Pro Lys Asp Ile Gly Ile Leu Val Val Asn Ser Ser Met 1.80 185 190 Phe Asn Pro Thr Pro Ser Leu Ser Ala Met Val Val Asn Thr Phe Lys 195 -200 Leu Arg Ser Asn Val Arg Ser Phe Asn Leu Gly Gly Met Gly Cys Ser 215 220 Ala Gly Val Ile Ala Ile Asp Leu Ala Lys Asp Leu Leu His Val His 230 235 Lys Asn Thr Tyr Ala Leu Val Val Ser Thr Glu Asn Ile Thr Tyr Asn 245 250 Ile Tyr Ala Gly Asp Asn Arg Ser Met Met Val Ser Asn Cys Leu Phe 260 265 Arg Val Gly Gly Ala Ala Ile Leu Leu Ser Asn Lys Pro Gly Asp Arg 280 Arg Arg Ser Lys Tyr Glu Leu Val His Thr Val Arg Thr His Thr Gly 295 300 Ala Asp Gly Lys Ser Phe Arg Cys Val Gln Gln Gly Asp Asp Glu Asn 310 315 Gly Lys Ile Gly Val Ser Leu Ser Lys Asp Ile Thr Asp Val Ala Gly 325 330 Arg Thr Val Lys Lys Asn Ile Ala Thr Leu Gly Pro Leu Ile Leu Pro 340 345 Leu Ser Glu Lys Leu Leu Phe Phe Val Thr Phe Met Gly Lys Lys Leu 355 360 365 Phe Lys Asp Lys Ile Lys His Tyr Tyr Val Pro Asp Phe Lys Leu Ala 375 380 Ile Asp His Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Val 390 395 Leu Glu Lys Asn Leu Ala Leu Ala Pro Ile Asp Val Glu Ala Ser Arg 405 410

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Ser Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr
                               425
Glu Leu Ala Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Lys
                           440
                                    445
Val Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val
                      455
                                          460
Trp Val Ala Leu Asn Asn Val Lys Ala Ser Thr Asn Ser Pro Trp Glu
                470
                               475
His Cys Ile Asp Arg Tyr Pro Val Lys Ile Asp Ser Asp Ser Gly Lys
              485
                                 490
Ser Glu Thr Arg Val Pro Asn Gly Arg Ser
           500
<210> 15
<211> 1521
<212> DNA
<213> Artificial Sequence
<220>
<223> 5' 342 bp from A. thaliana FAE1 (SEQ ID NO:1) and
      3' 1179 bp from B. napus elongase KCS (SEO ID
     NO:3), having a mutation at position 920;
     designated At114 G307D; hypothetical
<221> CDS
<222> (1)...(1518)
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Met Thr Ser Val Asn Val Lys Leu Leu Tyr Arg Tyr Val Leu Thr Asn
ttt ttc aac ctc tgt ttg ttc ccg tta acg gcg ttc ctc gcc gga aaa
                                                                    96
Phe Phe Asn Leu Cys Leu Phe Pro Leu Thr Ala Phe Leu Ala Gly Lys
god tot ogg ott acc ata aac gat otc cac aac tto ott too tat otc
                                                                   144
Ala Ser Arg Leu Thr Ile Asn Asp Leu His Asn Phe Leu Ser Tyr Leu
                           40
caa cac aac ctt ata aca qta act tta ctc ttt qct ttc act gtt ttc
                                                                   192
Gln His Asn Leu Ile Thr Val Thr Leu Leu Phe Ala Phe Thr Val Phe
                       55
ggt ttg gtt ctc tac atc gta acc cga ccc aat ccg gtt tat ctc gtt
                                                                   240
Gly Leu Val Leu Tyr Ile Val Thr Arg Pro Asn Pro Val Tyr Leu Val
                    70
gae tae teg tot tae ett eeg eea eeg eat ete aaa git agt gie tet
                                                                   288
Asp Tyr Ser Cys Tyr Leu Pro Pro Pro His Leu Lys Val Ser Val Ser
aaa gtc atg gat att ttc tac caa ata aga aaa gct gat act tct tca
                                                                   336
Lys Val Met Asp Ile Phe Tyr Gln Ile Arq Lys Ala Asp Thr Ser Ser
ogg aac ggc acg tgt gat gat teg teg tgg ctt gac ttc ttg agg aag
                                                                   384
Arg Asn Gly Thr Cys Asp Asp Ser Ser Trp Leu Asp Phe Leu Arg Lys
att caa gaa cgt tca ggt cta ggc gat gaa act cac ggg ccc gag ggg
                                                                   432
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Ile	Gln 130	Glu	Arg	Ser	Gly	Leu 135	Gly	Asp	Glu	Thr	His 140	Gly	Pro	Glu	Gly	
					ccc Pro 150											480
					att Ile											528
					gat Asp											576
					tcg Ser											624
					aga Arg											672
					att Ile 230											720
					ctt Leu											768
					aat Asn											816
					gct Ala											864
					gag Glu											912
					ttt Phe 310											960
												and to	-	ant	- contra	1008
ggc					agt Ser											1008
Gly	Lys	Ile gtt	Gly	Val 325 aaa		Leu	Ser gca	Lys	Asp 330 ttg	Ile ggt	Thr	Asp	Val att	Ala 335 ctt	Gly	1056
Gly cga Arg	Lys acg Thr	Ile gtt Val	aag Lys 340	Val 325 aaa Lys ctt	Ser	Leu ata Ile ttt	gca Ala	acg Thr 345	Asp 330 ttg Leu acc	Ile ggt Gly	Thr ccg Pro	Asp ttg Leu ggc	Val att Ile 350	Ala 335 ctt Leu	ccg Pro	

WO 01/94565 PCT/US01/18737 370 375 380 att gac cat tit tgt ata cat gcc gga ggc aga gcc gtg att gat gtg 1200 Ile Asp His Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Val 390 cta qaq aaq aac cta gec cta qea ceq ate gat qta gag gea tea aga 1248 Leu Glu Lys Asn Leu Ala Leu Ala Pro Ile Asp Val Glu Ala Ser Arg 405 tea acg tta cat aga ttt gga aac act tea tet age tea ata tgg tat 1296 Ser Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr 420 425 gag ttg gca tac ata gaa gca aaa gga agg atg aag aaa ggt aat aaa 1344 Glu Leu Ala Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Lys 440 gtt tgg cag att gct tta ggg tca ggc ttt aag tgt aac agt gca gtt 1392 Val Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val 455 tgg gtg gct cta aac aat gtc aaa gct tcg aca aat agt cct tgg gaa 1440 Trp Val Ala Leu Asn Asn Val Lys Ala Ser Thr Asn Ser Pro Trp Glu 470 475 cac tqc atc qac aga tac ccq qtc aaa att qat tct qat tca qqt aaq 1488 His Cys Ile Asp Arg Tyr Pro Val Lys Ile Asp Ser Asp Ser Gly Lys 485 490 tca gag act cgt gtc caa aac ggt cgg tcc taa 1521 Ser Glu Thr Arg Val Gln Asn Gly Arg Ser 500 <210> 16 <211> 506 <212> PRT <213> Artificial Sequence <223> 5' 114 amino acids from A. thaliana FAE1 (SEQ ID NO:2) and 3' 392 amino acids from B. napus elongase KCS (SEQ ID NO:4) having mutation at residue 307; designated At114 G307D; hypothetical <400> 16 Met Thr Ser Val Asn Val Lys Leu Leu Tyr Arg Tyr Val Leu Thr Asn Phe Phe Asn Leu Cys Leu Phe Pro Leu Thr Ala Phe Leu Ala Gly Lys Ala Ser Arg Leu Thr Ile Asn Asp Leu His Asn Phe Leu Ser Tvr Leu Gln His Asn Leu Ile Thr Val Thr Leu Leu Phe Ala Phe Thr Val Phe

28

Gly Leu Val Leu Tyr Ile Val Thr Arg Pro Asn Pro Val Tyr Leu Val

Asp Tyr Ser Cys Tyr Leu Pro Pro Pro His Leu Lys Val Ser Val Ser 85 Lys Val Met Asp ILe Phe Tyr Gln Ile Arg Lys Ala Asp Thr Ser Ser

105

70

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Arg Asn Gly Thr Cys Asp Asp Ser Ser Trp Leu Asp Phe Leu Arg Lys
                          120
Ile Gln Glu Arg Ser Gly Leu Gly Asp Glu Thr His Gly Pro Glu Gly
                      135
                                          140
Leu Leu Gln Val Pro Pro Arg Lys Thr Phe Ala Ala Ala Arg Glu Glu
                   150
                                      155
Thr Glu Gln Val Ile Ile Gly Ala Leu Glu Asn Leu Phe Lys Asn Thr
                                  170
              165
Asn Val Asn Pro Lys Asp Ile Gly Ile Leu Val Val Asn Ser Ser Met
                              185
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Phe Asn Pro Thr Pro Ser Leu Ser Ala Met Val Val Asn Thr Phe Lys
                          200
Leu Arg Ser Asn Val Arg Ser Phe Asn Leu Gly Gly Met Gly Cys Ser
                       215
Ala Gly Val Ile Ala Ile Asp Leu Ala Lys Asp Leu Leu His Val His
                   230
                                       235
Lys Asn Thr Tyr Ala Leu Val Val Ser Thr Glu Asn Ile Thr Tyr Asn
               245
                                  250
Ile Tyr Ala Gly Asp Asn Arg Ser Met Met Val Ser Asn Cys Leu Phe
           260
                              265
Arg Val Gly Gly Ala Ala Ile Leu Leu Ser Asn Lys Pro Gly Asp Arg
                           280
Arg Arg Ser Lys Tyr Glu Leu Val His Thr Val Arg Thr His Thr Gly
                       295
                                           300
Ala Asp Asp Lys Ser Phe Arg Cys Val Gln Gln Gly Asp Asp Glu Asn
                   310
                                      315
Cly Lys Ile Gly Val Ser Leu Ser Lys Asp Ile Thr Asp Val Ala Gly
               325
                                   330
Arg Thr Val Lys Lys Asn Ile Ala Thr Leu Gly Pro Leu Ile Leu Pro
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                              345
Leu Ser Glu Lys Leu Leu Phe Phe Val Thr Phe Met Gly Lys Lys Leu
                           360
                                              365
Phe Lys Asp Lys Ile Lys His Tyr Tyr Val Pro Asp Phe Lys Leu Ala
                       375
Ile Asp His Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Val
                   390
                                       395
Leu Glu Lys Asn Leu Ala Leu Ala Pro Ile Asp Val Glu Ala Ser Arg
               405
                                   410
Ser Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr
           420
                              425
Glu Leu Ala Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Lys
                          440
Val Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val
                      455
                                          460
Trp Val Ala Leu Asn Asn Val Lys Ala Ser Thr Asn Ser Pro Trp Glu
                   470
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His Cys Ile Asp Arg Tyr Pro Val Lys Ile Asp Ser Asp Ser Gly Lys
              485
Ser Glu Thr Arg Val Gln Asn Gly Arg Ser
          500
<210> 17
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<212> DNA
<213> Artificial Sequence
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<220>

<223> 5' 222 bp from A. thaliana FAE1 (SEQ ID NO:1) and 3' 1296 bp from B. napus elongase KCS (SEQ ID NO:3) having a mutation at position 917; designated At74 G306D; hypothetical

<221> CDS <222> (1)(1515)													
<400> 17 atg acg tcc gtt aac gtt aag ctc ctt tac cgt tac gtc tta acc aac Met Thr Ser Val Asn Val Lys Leu Leu Tyr Arg Tyr Val Leu Thr Asn	48												
ttt ttc aac ctc tgt ttg ttc ccg tta acg gcg ttc ctc gcc gga aaa Phe Phe Aen Leu Cys Leu Phe Pro Leu Thr Ala Phe Leu Ala Gly Lys 20 25 30	96												
gcc tct cgg ctt acc ata aac gat ctc cac aac ttc ctt tct tat ctc Ala Ser Arg Leu Thr Ile Asn Asp Leu His Asn Phe Leu Ser Tyr Leu 35 40 45	144												
caa cac aac ctt ata aca gta act tta ctc ttt gct ttc act gtt ttc Gln His Asn Leu Ile Thr Val Thr Leu Leu Phe Ala Phe Thr Val Phe 50 60	192												
ggt ttg gtt ctc tac atc gta acc oga ccc aaa ccg gtt tac ctc gtt Gly Leu Val Leu Tyr Ile Val Thr Arg Pro Lys Pro Val Tyr Leu Val 65 70 75 80	240												
gag tac toa tgc tac ott coa coa acg cat tgt aga toa agt atc toc Glu Tyr Ser Cys Tyr Leu Pro Pro Thr His Cys Arg Ser Ser Ile Ser 85 90 95	288												
aag gtc atg gat atc ttt tat caa gta aga aaa gct gat cct tct cgg Lys Val Met Asp Ile Phe Tyr Gln Val Arg Lys Ala Asp Pro Ser Arg $100 \hspace{1.5cm} 105 \hspace{1.5cm} 110$	336												
aac ggc acg tgc gat gac tog tcg tgg ctt gac ttc ttg agg aag att Asm Gly Thr Cys Asp Asp Ser Ser Trp Leu Asp Phe Leu Arg Lys Ile 115 120 125	384												
caa gaa cgt tca ggt cta ggc gat gaa act cac ggg ccc gag ggg ctg Gln Glu Arg Ser Gly Leu Gly Asp Glu Thr His Gly Pro Glu Gly Leu 130 135 140	432												
ctt cag gtc cct ccc cgg aag act ttt gcg gcg cgt gaa gag acg Leu Gln Val Pro Pro Arg Lys Thr Phe Ala Ala Ala Arg Glu Glu Thr 145 150 155 160	480												
gag caa gtt atc att ggt gcg cta gaa aat cta ttc aag aac acc aac Glu Gln Val Ile Ile Gly Ala Leu Glu Asn Leu Phe Lys Asn Thr Asn 165 170 175	528												
gtt aac oct aaa gat ata ggt ata ott gtg gtg aac toa agc atg ttt Val Asn Pro Lys Asp Ile Gly Ile Leu Val Val Asn Ser Ser Met Phe 180 185 190	576												
aat cca act cca tog ctc tcc gcg atg gtc gtt aac act ttc aag ctc Asn Pro Thr Pro Ser Leu Ser Ala Met Val Val Asn Thr Phe Lys Leu 195 200 205	624												
oga agc aac gta aga agc ttt aac ott ggt ggc atg ggt tgt agt goc Arg Ser Asn Val Arg Ser Phe Asn Leu Gly Gly Met Gly Cys Ser Ala 210 220	672												

WO 01/94565 PCT/US01/18737 gge gtt ata gee att gat eta gea aag gae tig tig eat gte eat aaa 720 Gly Val Ile Ala Ile Asp Leu Ala Lys Asp Leu Leu His Val His Lys aat acg tat get ett gtg gtg age aca gag aac ate act tat aac att 768 Asn Thr Tyr Ala Leu Val Val Ser Thr Glu Asn Ile Thr Tyr Asn Ile tac get ggt gat aat agg tee atg atg gtt tea aat tge ttg tte egt 816 Tyr Ala Gly Asp Asn Arg Ser Met Met Val Ser Asn Cys Leu Phe Arg 265 gtt ggt ggg gcc gct att ttg ctc tcc aac aag cct gga gat cgt aga 864 Val Gly Gly Ala Ala Ile Leu Leu Ser Asn Lys Pro Gly Asp Arg Arg egg tee aag tae gag eta gtt eac aeg gtt ega aeg eat aee gga get 912 Arg Ser Lys Tyr Glu Leu Val His Thr Val Arg Thr His Thr Gly Ala 295 960 gac gac aag tet ttt egt tge gtg caa caa gga gac gat gag aac gge Asp Asp Lys Ser Phe Arg Cys Val Gln Gln Gly Asp Asp Glu Asn Gly 310 315 aaa atc qqa qtq agt ttq tcc aaq gac ata acc gat gtt gct qgt cqa 1008 Lys Ile Gly Val Ser Leu Ser Lys Asp Ile Thr Asp Val Ala Gly Arg 330 acg gtt aag aaa aac ata gca acg ttg ggt ccg ttg att ctt ccg tta 1056 Thr Val Lys Lys Asn Ile Ala Thr Leu Gly Pro Leu Ile Leu Pro Leu 345 age gag aaa ett ett tte gtt ace tte atg gge aag aaa ett tte 1104 Ser Glu Lys Leu Leu Phe Phe Val Thr Phe Met Gly Lys Lys Leu Phe 360 aaa gat aaa atc aaa cat tac tac gtc cog gat ttc aaa ctt gct att 1152 Lys Asp Lys Ile Lys His Tyr Tyr Val Pro Asp Phe Lys Leu Ala Ile 375 gac cat tit tgt ata cat gcc gga ggc aga gcc gtg att gat gtg cta 1200 Asp His Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Val Leu 390 gag aag aac cta gee cta gea ceg atc gat gta gag gea tea aga tea 1248 Glu Lys Asn Leu Ala Leu Ala Pro Ile Asp Val Glu Ala Ser Arg Ser 405 acg tta cat aga ttt qga aac act tca tct agc tca ata tqq tat gag 1296 Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr Glu 425 ttg gca tac ata gaa gca aaa gga agg atg aag aaa ggt aat aaa gtt 1344 Leu Ala Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Lys Val 440 tgg cag att get tta ggg tea ggc ttt aag tgt aac agt gea gtt tgg 1392 Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val Trp 455

1440

gtg get eta aac aat gte aaa get teg aca aat agt eet tgg gaa cac

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Val Ala Leu Asn Asn Val Lys Ala Ser Thr Asn Ser Pro Trp Glu His 470

tgc atc gac aga tac ccg gtc aaa att gat tct gat tca ggt aag tca Cys Ile Asp Arg Tyr Pro Val Lys Ile Asp Ser Asp Ser Gly Lys Ser

gag act egt gte caa aac ggt egg tee taa 1518 Glu Thr Arg Val Gln Asn Gly Arg Ser 500

<210> 18 <211> 505 <212> PRT

<213> Artificial Sequence

<223> 5' 74 amino acids from A. thaliana FAE1 (SEQ ID NO:2) and 3' 431 amino acids from B. napus elongase KCS (SEQ ID NO:4) having a mutation at residue 306; designated At74 G306D; hypothetical

Met Thr Ser Val Asn Val Lys Leu Leu Tyr Arg Tyr Val Leu Thr Asn Phe Phe Asn Leu Cys Leu Phe Pro Leu Thr Ala Phe Leu Ala Gly Lys Ala Ser Arg Leu Thr Ile Asn Asp Leu His Asn Phe Leu Ser Tyr Leu Gln His Asn Leu Ile Thr Val Thr Leu Leu Phe Ala Phe Thr Val Phe Gly Leu Val Leu Tyr Ile Val Thr Arg Pro Lys Pro Val Tyr Leu Val Glu Tyr Ser Cys Tyr Leu Pro Pro Thr His Cys Arg Ser Ser Ile Ser Lys Val Met Asp Ile Phe Tyr Gln Val Arg Lys Ala Asp Pro Ser Arg 105 Asn Gly Thr Cys Asp Asp Ser Ser Trp Leu Asp Phe Leu Arg Lys Ile 120 Gln Glu Arg Ser Gly Leu Gly Asp Glu Thr His Gly Pro Glu Gly Leu 135 140 Leu Gln Val Pro Pro Arg Lys Thr Phe Ala Ala Ala Arg Glu Glu Thr 150 1.55 Glu Gln Val Ile Ile Gly Ala Leu Glu Asn Leu Phe Lys Asn Thr Asn 170 Val Asn Pro Lys Asp Ile Gly Ile Leu Val Val Asn Ser Ser Met Phe 185 Asn Pro Thr Pro Ser Leu Ser Ala Met Val Val Asn Thr Phe Lys Leu 200 Arg Ser Asn Val Arg Ser Phe Asn Leu Gly Gly Met Gly Cys Ser Ala 215 Gly Val Ile Ala Ile Asp Leu Ala Lys Asp Leu Leu His Val His Lys 230 235 Asn Thr Tyr Ala Leu Val Val Ser Thr Glu Asn Ile Thr Tyr Asn Ile 250 Tyr Ala Gly Asp Asn Arg Ser Met Met Val Ser Asn Cys Leu Phe Arg 265 Val Gly Gly Ala Ala Ile Leu Leu Ser Asn Lys Pro Gly Asp Arg Arg 280 Arg Ser Lys Tyr Glu Leu Val His Thr Val Arg Thr His Thr Gly Ala

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295
                                            300
Asp Asp Lys Ser Phe Arg Cys Val Gln Gln Gly Asp Asp Glu Asn Gly
                 310
                                      315
Lys Ile Gly Val Ser Leu Ser Lys Asp Ile Thr Asp Val Ala Gly Arg
                                   330
Thr Val Lys Lys Asn Ile Ala Thr Leu Gly Pro Leu Ile Leu Pro Leu
                               345
Ser Glu Lys Leu Leu Phe Phe Val Thr Phe Met Gly Lys Lys Leu Phe
                           360
Lys Asp Lys Ile Lys His Tyr Tyr Val Pro Asp Phe Lys Leu Ala Ile
                       375
                                           380
Asp His Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Val Leu
                   390
                                       395
Glu Lys Asn Leu Ala Leu Ala Pro Ile Asp Val Glu Ala Ser Arg Ser
               405
                                   410
Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr Glu
                                425
Leu Ala Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Lys Val
                           440
Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val Trp
                        455
Val Ala Leu Asn Asn Val Lys Ala Ser Thr Asn Ser Pro Trp Glu His
                   470
                                       475
Cys Ile Asp Arg Tyr Pro Val Lys Ile Asp Ser Asp Ser Gly Lys Ser
               485
                                  490
Glu Thr Arg Val Gln Asn Gly Arg Ser
           500
<210> 19
<211> 1521
<212> DNA
<213> Artificial Sequence
<223> 5' 342 bp from A. thaliana FAE1 (SEQ ID NO:1) and
      3' 1179 bp from B. napus elongase KCS (SEQ ID
      NO:3) having mutations at positions 271, 272, 275
      and 920; designated At114 L91C K92R G307D;
      hypothetical
<221> CDS
<222> (1) ... (1518)
<400> 19
atg acg tcc gtt aac gtt aag ctc ctt tac cgt tat gtc tta acc aac
                                                                     48
Met Thr Ser Val Asn Val Lys Leu Leu Tyr Arg Tyr Val Leu Thr Asn
                                     10
ttt tte aac ete tot ttg tte eeg tta acg geg tte ete gee gga aaa
                                                                      96
Phe Phe Asn Leu Cvs Leu Phe Pro Leu Thr Ala Phe Leu Ala Gly Lys
                                 25
goe tot egg ott ace ata aac gat etc cac aac tte ott toc tat etc
                                                                     144
Ala Ser Arg Leu Thr Ile Asn Asp Leu His Asn Phe Leu Ser Tyr Leu
caa cac aac ctt ata aca gta act tta ctc ttt gct ttc act gtt ttc
                                                                     192
Gln His Asn Leu Ile Thr Val Thr Leu Leu Phe Ala Phe Thr Val Phe
ggt ttg gtt etc tac atc gta acc ega ecc aat eeg gtt tat etc gtt
                                                                    240
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Glv Leu Val Leu Tyr Ile Val Thr Arg Pro Asn Pro Val Tyr Leu Val

Gly 65	Leu	Val	Leu	Tyr	Ile 70	Val	Thr	Arg	Pro	Asn 75	Pro	Val	Тук	Leu	Val 80	
					ctt Leu											288
					ttc Phe											336
					gat Asp											384
					ggt Gly											432
					ccc Pro 150											480
					att Ile											528
aac Asn	gtt Val	aac Asn	cct Pro 180	aaa Lys	gat Asp	ata Ile	ggt Gly	ata Ile 185	ctt Leu	gtg Val	gtg Val	aac Asn	tca Ser 190	agc Ser	atg Met	576
					tcg Ser											624
					aga Arg											672
					att Ile 230											720
aaa Lys	aat Asn	acg Thr	tat Tyr	gct Ala 245	ctt Leu	gtg Val	gtg Val	agc Ser	aca Thr 250	gag Glu	aac Asn	atc Ile	act Thr	tat Tyr 255	aac Asn	768
					aat Asn											816
					gct Ala											864
					gag Glu											912
					ttt Phe											960

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305		310		315			320	
ggc aaa a Gly Lys 1	Ile Gly		Lys 2					1008
cga acg g								1056
tta age g Leu Ser (Val 7		Met			1104
ttc aaa g Phe Lys J 370								1152
att gac (Ile Asp H 385					Ala			1200
cta gag a Leu Glu I	Lys Asn		Pro :					1248
tca acg t Ser Thr I								1296
gag ttg g Glu Leu 1								1344
gtt tgg (Val Trp (450								1392
tgg gtg g Trp Val 1 465					Asn			1440
cac tgc a	Ile Asp		Lys :					1488
tca gag a Ser Glu 1								1521

<210> 20

<211> 506

<212> PRT

<213> Artificial Sequence

<220:

<223> 5' 114 amino acids from A. thaliana FAE1 (SEQ ID NO:2) and 3' 392 amino acids from B. napus elongase KCS (SEQ ID NO:4) having mutations at positions 91, 92 and 307; designated Atl14 L91C

K92R G307D; hypothetical

-404)> 20														
			Val	3	77-7		T	*	m	7	m	77-7	T	m1	7
2				5					10		-			15	
Phe	Phe	Asn	Leu 20	Cys	Leu	Phe	Pro	Leu 25	Thr	Ala	Phe	Leu	Ala 30	Gly	Lys
Ala	ser	Arg 35	Leu	Thr	Ile	Asn	Asp 40	Leu	His	Asn	Phe	Leu 45	Ser	Tyr	Leu
Gln	His 50	Asn	Leu	Ile	Thr	Val 55	Thr	Leu	Leu	Phe	Ala 60	Phe	Thr	Val	Phe
Gly	Leu	Val	Leu	тут	Ile 70	Val	Thr	Arg	Pro	Asn 75	Pro	Val	Tyr	Leu	Val 80
Asp	Tyr	Ser	Cys	Tyr 85	Leu	Pro	Pro	Pro	His 90	Cys	Arg	Val	Ser	Val 95	Ser
Lys	Val	Met	Asp	Ile	Phe	Tyr	Gln	Ile 105	Arg	Lys	Ala	Asp	Thr	Ser	Ser
Arg	Asn	Gly 115	Thr	Сув	Asp	Asn	Ser 120		Trp	Leu	Asp	Phe 125	Leu	Arg	Lys
Ile	Gln 130	Glu	Arg	Ser	Gly	Leu 135	Gly	Asp	Glu	Thr	His	Gly	Pro	Glu	Gly
Leu 145	Leu	Gln	Val	Pro	Pro	Arg	ГЛЗ	Thr	Phe	Ala 155	Ala	Ala	Arg	Glu	Glu 160
Thr	Glu	Gln	Val	Ile 165	Ile	Gly	Ala	Leu	Glu 170	Asn	Leu	Phe	Lys	Asn 175	
Asn	Val	Asn	Pro 180	ГЛЗ	Asp	Ile	Gly	Ile 185	Leu	Val	Val	Asn	Ser 190	Ser	Met
Phe	Asn	Pro 195	Thr	Pro	Ser	Leu	Ser 200	Ala	Met	Val	Val	Asn 205	Thr	Phe	Lys
	210		Asn			215					220				
Ala 225	Gly	Val	Ile	Ala	Ile 230	Asp	Leu	Ala	Lys	Asp 235	Leu	Leu	His	Val	His 240
Lys	Asn	Thr	Tyr	Ala 245	Leu	Val	Val	ser	Thr 250	Glu	Asn	Ile	Thr	Tyr 255	Asn
Ile	Tyr	Ala	Gly 260	Asp	Asn	Arg	Ser	Met 265	Met	Val	Ser	Asn	Cys 270	Leu	Phe
-		275	Gly				280					285	_	_	-
_	290		Lys			295					300				_
Ala 305	Asp	Asp	Lys	Ser	Phe 310	Arg	Cys	Val	Gln	Gln 315	Gly	Asp	Asp	Glu	Asn 320
Gly	Lys	Ile	Gly	Val 325	Ser	Leu	Ser	Lys	Asp 330	Ile	Thr	Asp	Val	Ala 335	Gly
			Lys 340					345					350		
Leu	Ser	Glu 355	Lys	Leu	Leu	Phe	Phe 360	Val	Thr	Phe	Met	Gly 365	Lys	Lys	Leu
	370		Lys			375					380				
Ile 385	Asp	His	Phe	Cys	Ile 390	His	Ala	Gly	Gly	Arg 395	Ala	Val	Ile	Asp	Val
Leu	Glu	Lys	Asn	Leu 405	Ala	Leu	Ala	Pro	Ile	Asp	Val	Glu	Ala	Ser 415	Arg
Ser	Thr	Leu	His 420	Arg	Phe	Gly	Asn	Thr 425	Ser	Ser	Ser	Ser	Ile 430	Trp	Tyr
Glu	Leu	Ala 435	Tyr	Ile	Glu	Ala	Lys 440	Gly	Arg	Met	Lys	Lys 445	Gly	Asn	Lys
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	eu Ile Thr Va		ttt gct ttc act Phe Ala Phe Thr 60	
			aat cog gtt tat Asn Pro Val Tyr 75	
			ctc aga gtt agt Leu Arg Val Ser	
	p Ile Phe Ty		aaa gct gat act Lys Ala Asp Thr 110	
			ctt gac ttc ttg Leu Asp Phe Leu 125	
		u Gly Asp Glu	act cac ggg cec Thr His Gly Pro 140	
			gcg gcg gcg cgt Ala Ala Ala Arg 155	

acg gag o				Leu								528
aac gtt a Asn Val A												576
ttt aat o Phe Asn I				Ala								624
ctc cga a Leu Arg S 210												672
gcc ggc g Ala Gly V 225												720
aaa aat a Lys Asn 1				Ser								768
att tac o												816
cgt gtt g Arg Val 0	ggt ggg 31y Gly 275	gcc gct Ala Ala	att ttg Ile Leu 280	Leu	tcc Ser	aac Asn	aag Lys	cct Pro 285	gga Gly	gat Asp	cgt Arg	864
aga cgg t Arg Arg S 290												912
get gae g Ala Asp A 305												960
ggc aaa a Gly Lys 1												1008
cga acg o												1056
tta agc g Leu Ser G												1104
ttc aaa g Phe Lys A 370												1152
att gac o Ile Asp E 385												1200

WO 01/94565 PCT/US01/18737 cta gag aag aac cta goc cta goa cog atc gat gta gag goa toa aga Leu Glu Lys Asn Leu Ala Leu Ala Pro Ile Asp Val Glu Ala Ser Arg tca acg tta cat aga ttt gga aac act tca tct agc tca ata tgg tat 1296 Ser Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr gag ttg goa tac ata gaa goa aaa gga agg atg aag aaa ggt aat aaa 1344 Glu Leu Ala Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Lys 435 440 gtt tgg cag att gct tta ggg tca ggc ttt aag tgt aac agt gca gtt 1392 Val Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val 450 tgg gtg gct cta aac aat gtc aaa gct tcg aca aat agt cet tgg gaa 1440 Trp Val Ala Leu Asn Asn Val Lys Ala Ser Thr Asn Ser Pro Trp Glu 475 cac tgc atc gac aga tac ccg gtc aaa att gat tct gat tca ggt aag 1488 His Cys Ile Asp Arg Tyr Pro Val Lys Ile Asp Ser Asp Ser Gly Lys 485 490 1521 tca gag act cgt qtc caa aac ggt cgg tcc taa Ser Glu Thr Arg Val Gln Asn Gly Arg Ser 500 <210> 22 <211> 506 <212> PRT <213> Artificial Sequence <223> 5' 114 amino acids from A. thaliana FAE1 (SEQ ID NO:2) and 3' 392 amino acids from B. napus elongase KCS (SEO ID NO:4) having mutations at positions 92 and 307; designated At114 K92R G307D; hypothetical

<400> 22

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Leu Leu Gln Val Pro Pro Arg Lys Thr Phe Ala Ala Ala Arg Glu Glu

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145
                   150
                                       155
Thr Glu Gln Val Ile Ile Gly Ala Leu Glu Asn Leu Phe Lys Asn Thr
               165
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Asn Val Asn Pro Lys Asp Ile Gly Ile Leu Val Val Asn Ser Ser Met
           180
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Phe Asn Pro Thr Pro Ser Leu Ser Ala Met Val Val Asn Thr Phe Lys
                           200
Leu Arg Ser Asn Val Arg Ser Phe Asn Leu Gly Gly Met Gly Cys Ser
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                                           220
Ala Gly Val Ile Ala Ile Asp Leu Ala Lys Asp Leu Leu His Val His
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                   230
Lys Asn Thr Tyr Ala Leu Val Val Ser Thr Glu Asn Ile Thr Tyr Asn
                                   250
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Ile Tyr Ala Gly Asp Asn Arg Ser Met Met Val Ser Asn Cys Leu Phe
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Arg Val Gly Gly Ala Ala Ile Leu Leu Ser Asn Lys Pro Gly Asp Arg
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Arg Arg Ser Lys Tyr Glu Leu Val His Thr Val Arg Thr His Thr Gly
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Ala Asp Asp Lys Ser Phe Arg Cys Val Gln Gln Gly Asp Asp Glu Asn
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Gly Lys Ile Gly Val Ser Leu Ser Lys Asp Ile Thr Asp Val Ala Gly
                                   330
Arg Thr Val Lys Lys Asn Ile Ala Thr Leu Gly Pro Leu Ile Leu Pro
                               345
Leu Ser Glu Lys Leu Leu Phe Phe Val Thr Phe Met Gly Lys Lys Leu
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                                               365
Phe Lys Asp Lys Ile Lys His Tyr Tyr Val Pro Asp Phe Lys Leu Ala
                       375
Ile Asp His Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Val
                   390
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Leu Glu Lys Asn Leu Ala Leu Ala Pro Ile Asp Val Glu Ala Ser Arg
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Ser Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr
           420
                               425
Glu Leu Ala Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Lys
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Val Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val
   450
                       455
                                           460
Trp Val Ala Leu Asn Asn Val Lys Ala Ser Thr Asn Ser Pro Trp Glu
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Ser Glu Thr Arg Val Gln Asn Gly Arg Ser
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     NO:3): designated At254
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40

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				acc Thr												14	4
				ata Ile												19	2
				tac Tyr												24	0
				tac Tyr 85												28	B
				att Ile												33	16
				tgt Cys												38	14
				tca Ser												43	12
				cca Pro												48	10
				atc Ile 165												52	8
				aga Arg												57	6
				cct Pro												62	4
				atc Ile												67	2
				g c c Ala												72	0
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245	250)	255
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		aac aag oot gga Asn Lys Pro Gly 285	
		gtt ega acg cat Val Arg Thr His 300	
		caa gga gac gat Gln Gly Asp Asp 315	
		ata acc gat gtt The Thr Asp Val	
		ggt ccg ttg att Gly Pro Leu Ile 350	
		ttc atg ggc aag Phe Met Gly Lys 365	
		ccg gat ttc aaa Pro Asp Phe Lys 380	
		aga gcc gtg att Arg Ala Val Ile 395	
		gat gta gag gca Asp Val Glu Ala	
		tct agc tca ata Ser Ser Ser Ile 430	
		atg aag aaa ggt Met Lys Lys Gly 445	
		aag tgt aac agt Lys Cys Asn Ser 460	
		aca aat agt cct Thr Asn Ser Pro 475	
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Leu	Ser	Glu 355	Lys	Leu	Leu	Phe	Phe	Val	Thr	Phe	Met	Gly	Lys	Lys	Leu	
Phe	Lys 370	Asp	Lys	Ile	Lys	His 375	Tyr	Tyr	Val	Pro	Asp 380	Phe	Lys	Leu	Ala	
Ile 385	Asp	His	Phe	Cys	Ile 390	His	Ala	Gly	Gly	Arg 395	Ala	Val	Ile	Asp	Val	
	Glu	Lys	Asn	Leu 405		Leu	Ala	Pro	Ile 410		Val	Glu	Ala	Ser 415		
Ser	Thr	Leu	His 420		Phe	Gly	Asn	Thr 425		Ser	ser	Ser	Ile 430		Tyr	
Glu	Leu	Ala 435		Ile	Glu	Ala	Lys	Gly	Arg	Met	ьуѕ	Lys 445		Asn	Lys	
Val	Trp		Ile	Ala	Leu	Gly 455		Gly	Phe	Lys	Cys		Ser	Ala	Val	
Trp	Val	Ala	Leu	Asn	Asn 470	Val	Lys	Ala	Ser	Thr 475	Asn	Ser	Pro	Trp	Glu 480	
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Ser	Glu	Thr	Arg 500	Val	Pro	Asn	Gly	Arg 505	Ser							
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	144):3/,	: aes	signa	atea	MCT.	13									
	L> CI	os	. (151		atea	MCI	/3									
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	100	10	05	110	
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				s agt oot gag g r Ser Pro Glu G D	
				g tca cgt gaa g a Ser Arg Glu G 1	
				a ttc aag aac a 1 Phe Lys Asn T 175	
		Ile Gly I		g aac tca agc a l Asn Ser Ser N 190	
				t aac act ttc a l Asn Thr Phe I 205	
				c atg ggt tgt a y Met Gly Cys s O	
				g ttg cat gtc o u Leu His Val E 2	
				c atc act tat a n Ile Thr Tyr A 255	
		Arg Ser Me		a aat tgc ttg t r Asn Cys Leu F 270	
				g cct gga gat o s Pro Gly Asp A 285	
aga cgg tcc Arg Arg Ser 290	aag tac gag Lys Tyr Glu	cta gtt ca Leu Val Hi 295	ac acg gtt cgs is Thr Val Arg 300	a acg cat acc g g Thr His Thr G	ga 912 Ely
				a gac gat gag a y Asp Asp Glu A 3	
				c gat gtt gct g r Asp Val Ala G 335	
		Ile Ala Th		g ttg att ctt c o Leu Ile Leu F 350	

	agc Ser										1104
	aaa Lys 370										1152
	gac Asp										1200
	gag Glu										1248
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	ttg Leu										1344
	tgg Trp 450										1392
	gtg Val										1440
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<400> 26

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Gln	His 50	Asn	Leu	Ile	Thr	Val 55	Thr	Leu	Leu	Phe	Ala 60	Phe	Thr	Val	Phe
Gly	Leu	Val	Leu	Tyr	Ile 70	Val	Thr	Arg	Pro	Asn 75	Pro	Val	Tyr	Leu	Val
Asp	Tyr	Ser	Cys	Tyr 85		Pro	Pro	Pro	His 90		Lys	Val	Ser	Val. 95	
Lys	Val	Met	Asp		Phe	Tyr	Gln	Ile 105		Lys	Ala	Asp	Thr		Ser
Arg	Asn	Val		Суз	Asp	Asp	Pro 120		ser	Leu	Asp	Phe 125		Arg	Lys
Ile	Gln 130	Glu	Arg	Ser	Gly	Leu 135	Gly	Asp	Glu	Thr	Tyr 140	Ser	Pro	Glu	Gly
Leu 145	Ile	His	Val	Pro	Pro	Arg	Lys	Thr	Phe	Ala 155	Ala	Ser	Arg	Glu	Glu 160
	Glu	Lys	Val	Ile 165		Gly	Ala	Leu	Glu 170		Leu	Phe	Lys	Asn 175	
Asn	Val	Asn	Pro	Lуs	Asp	Ile	Gly	Ile 185	Leu	Val	Val	Asn	Ser	Ser	Met
Phe	Asn	Pro		Pro	Ser	Leu	Ser 200		Met	Val	Val	Asn 205		Phe	Lys
Leu	Arg 210		Asn	Val	Arg	Ser 215		Asn	Leu	Gly	Gly 220		Gly	Cys	Ser
Ala 225	Gly	Val	Ile	Ala	Ile 230		Leu	Ala	Lys	Asp 235		Leu	His	Val	His 240
Lys	Asn	Thr	Tyr	Ala 245		Val	Val	Ser	Thr 250		Asn	Ile	Thr	Tyr 255	
Ile	Tyr	Ala	Gly 260	qaA	Asn	Arg	Ser	Met 265	Met	Val	ser	Asn	Cys 270	Leu	Phe
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Ser	Thr	Leu	His		Phe	Gly	Asn	Thr 425		Ser	Ser	Ser	Ile 430		Tyr
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Trp	Val	Ala	Leu	Asn	Asn 470		Lys	Ala	Ser	Thr 475		Ser	Pro	Trp	Glu 480
	Cys	Ile	Asp	Arg		Pro	Val	Lys	Ile 490		Ser	Asp	Ser	Gly 495	
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got tat egg oft acc ata gac gat oft cac cac tha tac tat too tat Ala Tyr Arg Leu Thr Tle Asp Leu His His Leu Tyr Tyr Ser Tyr $_{\rm 35}$ $_{\rm 45}$	144
ctc caa cac aac ctc ata acc atc gct cca ctc ttt gcc ttc acc gtt Leu Gln His Asn Leu Ile Thr Ile Ala Pro Leu Phe Ala Phe Thr Val 50 55 60	192
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ctg ctt cag gto cct ccc cgg aag act ttt gcg gcg gcg cgt gaa gag $$\rm 4t$ Leu Leu Glu Val Pro Pro Arg Lys Thr Phe Ala Ala Ala Arg Glu Glu 145 150 150 150	180
acg gag caa gtt atc att ggt gcg cta gaa aat cta ttc aag aac acc Thr Glu Gln Val Ile Ile Gly Ala Leu Glu Asn Leu Phe Lys Asn Thr 165 170 175	528
aaa gtt aac oct aga gag att ggt ata ott gtg gtg aac toa ago atg Lys Val Asm Foo Arg Glu Ile Gly Ile Leu Val Val Asm Ser Ser Met 180 180 190	576

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		aaa Lys						672
		att Ile 230						720
		ctt Leu						768
		aat Asn						816
		geg Ala						864
		aag Lys						912
		ttt Phe 310						960
		Cya						1008
		aat Asn						1056
		ctt Leu						1104
		aag Lys						1152
		att Ile 390						1200
		gga Gly						1248
		ttt Phe						1296

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Glu Leu Ala Tyr lle Glu Ala Lys Gly Arg Met Lys Lys Gly Arn Lys
435

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Ala Tyr Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val
450

450

PCT/US01/18737

1344

1344

1344

1345

1346

1392

tgg gtg gct cta ogc aat gtc aag gca tog gca aat agt cct tgg caa
Ttp Val Ala Leu Arg Asn Val Lys Ala Ser Ala Asn Ser Pro Trp Gln
475 480
cat tgc atc gat aga tat cog gtt aaa att gat tct gat ttg tca aag 1488

tca aag act cat gtc caa aac ggt cgg tcc taa Ser Lys Thr His Val Gln Asn Gly Arg Ser 500 505

<210> 28 <211> 506

<212> PRT <213> Artificial Sequence

<220>

<223> 5' 176 amino acids from B. napus elongase KCS (SEQ ID NO:4) and 3' 330 amino acids from A. thaliana FABI (SEC ID NO:2); designated Bn176

<400> 28

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Ala Gly Val Ile Ala Ile Asp Leu Ala Lys Asp Leu Leu His Val His

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230
                                      235
Lys Asn Thr Tyr Ala Leu Val Val Ser Thr Glu Asn Ile Thr Gln Gly
               245 250 255
Ile Tyr Ala Gly Glu Asn Arg Ser Met Met Val Ser Asn Cys Leu Phe
           260
                              255
Arg Val Gly Gly Ala Ala Ile Leu Leu Ser Asn Lys Ser Gly Asp Arg
                           280
Arg Arg Ser Lys Tyr Lys Leu Val His Thr Val Arg Thr His Thr Gly
                       295
Ala Asp Asp Lys Ser Phe Arg Cys Val Gln Gln Glu Asp Asp Glu Ser
                   310
                                      315
Gly Lys Ile Gly Val Cys Leu Ser Lys Asp Ile Thr Asn Val Ala Gly
               325
                                  330
Thr Thr Leu Thr Lys Asn Ile Ala Thr Leu Gly Pro Leu Ile Leu Pro
           340
                               345
Leu Ser Glu Lys Phe Leu Phe Phe Ala Thr Phe Val Ala Lys Lys Leu
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Leu Lys Asp Lys Ile Lys His Tyr Tyr Val Pro Asp Phe Lys Leu Ala
                       375
Val Asp His Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Glu
                   390
                                       395
Leu Glu Lys Asn Leu Gly Leu Ser Pro Ile Asp Val Glu Ala Ser Arg
               405
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Ser Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr
                               425
Glu Leu Ala Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Lys
Ala Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val
                       455
Trp Val Ala Leu Arg Asn Val Lys Ala Ser Ala Asn Ser Pro Trp Gln
                   470
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His Cys Ile Asp Arg Tyr Pro Val Lys Ile Asp Ser Asp Leu Ser Lys
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Ser Lys Thr His Val Gln Asn Gly Arg Ser
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<210> 29
<211> 1521
<212> DNA
<213> Artificial Sequence
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     3' 324 bp from B. napus elongase KCS (SEQ ID
     NO:3); designated At399
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ttt ttc aac ctc tgt ttg ttc ccg tta acg gcg ttc ctc gcc gga aaa
                                                                     96
Phe Phe Asn Leu Cys Leu Phe Pro Leu Thr Ala Phe Leu Ala Gly Lys
                                25
                                                                   144
gee tet egg ett ace ata aac gat etc cac aac tte ett tee tat etc
Ala Ser Arg Leu Thr Ile Asn Asp Leu His Asn Phe Leu Ser Tyr Leu
        35
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									ctc Leu							192
ggt Gly 65	ttg Leu	gtt Val	ct c Leu	tac Tyr	atc Ile 70	gta Val	acc Thr	cga Arg	ccc Pro	aat Asn 75	ccg Pro	gtt Val	tat Tyr	ctc Leu	gtt Val 80	240
									cat His 90							288
									aga Arg							336
									tog Ser							384
									gag Glu							432
									ttt Phe							480
									gaa Glu 170							528
									ctt Leu							576
									atg Met							624
									cta Leu							672
									aaa Lys							720
									act Thr 250							768
									atg Met							816
									tct Ser							864

WO 01/94565 PCT/US01/18737

				cac His				912	
				gtg Val				960	
				aag Lys				1008	
				aca Thr 345				1056	
				gct Ala				1104	
				tat Tyr				1152	
				gga Gly				1200	
				ccg Pro				1248	
				act Thr 425				1296	
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				cgg Arg 505	taa			1521	

<210> 30 <211> 506

<211> 506 <212> PRT

<213> Artificial Sequence

<220>

<223> 5' 399 amino acids from A. thaliana FAE1 (SEQ ID NO:2) and 3' 107 amino acids from B. napus elongase KCS (SEQ ID NO:4); designated At399

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<222> (0)...(0)

<223> Xaa = Pro or Gln

<400> 30

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Leu	Glu	Lys	Asn	Leu 405	Ala	Leu	Ala	Pro	Ile 410	Asp	Val	Glu	Ala	Ser	Arg	
Ser	Thr	Leu	His 420		Phe	Gly	Asn	Thr 425		Ser	Ser	Ser	Ile 430	Trp	Tyr	
31u	Leu	Ala 435	Tyr	Ile	Glu	Ala	Lys 440	Gly	Arg	Met	гла	Lys 445	Gly	Asn	Lys	
	450					Gly 455					460					
65					470	Val	_			475					480	
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	> DN > Ar		lcial	L Sec	queno	ce							,			
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	NC	(2:0	and	3 ' 3	324 h	p fi	om 2						ID			
	.> CI			-												
			. (15)	L8)												
atg	> 31 acg	tcc	att	aac	gtt	aag Lys	ctc	ctt	tac	cat	tac	gtc	ata	acc	aac	48
1	TILL	Per	116	5	vai	Бур	Deu	Deu	10	nro	-3-	val	110	15		
						ttt Phe										96
			20					25					30			
		Arg				gac Asp	Asp					Tyr				144
		35	224	aha	2#2	acc	40	e e t	002	ata		45	tta	200	art.	192
						Thr 55										1,72
tc	qgt	tog	gtt	ata	tac	atc	gca	acc	cgg	ccc	aaa	ccg	gtt	tac	ctc	240
						Ile										
						ctt										288
aı	GIU	ıyr	ser	85	TYL	Leu	PIO	Pro	90	HIS	Cys	Arg	ser	95	116	
						ttt Phe										336
	-,-		100				-14	105		3	-,5		110			
						gaç Asp										384
-		115		-			120					125				

					ggt Gly											432
					ccc Pro 150											480
					att Ile											528
					gat Asp											576
					tcg Ser											624
					aga Arg											672
					att Ile 230											720
					ctt Leu											768
att Ile	tac Tyr	gct Ala	ggt Gly 260	gat Asp	aat Asn	agg Arg	tcc Ser	atg Met 265	atg Met	gtt Val	tca Ser	aat Asn	tgc Cys 270	ttg Leu	ttc Phe	816
					gct Ala											864
					gag Glu											912
					ttt Phe 310											960
					agt Ser											1008
					aac Asn											1056
					ctt Leu											1104
tte																1152

Phe	Lys 370	Asp	Lys	Ile	Lys	His 375	Tyr	Tyr	Val	Pro	Asp 380	Phe	Lys	Leu	Ala	
				tgt Cys												1200
				tta Leu 405												1248
				aga Arg												1296
				ata Ile												1344
				gct Ala												1392
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				gtc Val						taa						1521
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)> 3: Thr		Ile	Asn 5	Val	Lys	Leu	Leu	Tyr 10	His	Tyr	Val	Ile	Thr	Asn	
			20	Cys				25					30			

105

1.00

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Arg Asn Gly Thr Cys Asp Asp Ser Ser Trp Leu Asp Phe Leu Arg Lys
                            120
       115
Ile Gln Glu Arg Ser Gly Leu Gly Asp Glu Thr His Gly Pro Glu Gly
                        135
                                            140
Leu Leu Gln Val Pro Pro Arg Lys Thr Phe Ala Ala Ala Arg Glu Glu
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Thr Glu Gln Val Ile Ile Gly Ala Leu Glu Asn Leu Phe Lys Asn Thr
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                                    170
                                                        175
Asn Val Asn Pro Lys Asp Ile Gly Ile Leu Val Val Asn Ser Ser Met
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                                185
                                                   190
Phe Asn Pro Thr Pro Ser Leu Ser Ala Met Val Val Asn Thr Phe Lys
                            200
Leu Arg Ser Asn Val Arg Ser Phe Asn Leu Gly Gly Met Gly Cys Ser
                        215
                                            220
Ala Gly Val Ile Ala Ile Asp Leu Ala Lys Asp Leu Leu His Val His
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                                        235
Lys Asn Thr Tyr Ala Leu Val Val Ser Thr Glu Asn Ile Thr Tyr Asn
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Ile Tyr Ala Gly Asp Asn Arg Ser Met Met Val Ser Asn Cys Leu Phe
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                                265
                                                    270
Arg Val Gly Gly Ala Ala Ile Leu Leu Ser Asn Lys Pro Gly Asp Arg
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Arg Arg Ser Lys Tyr Glu Leu Val His Thr Val Arg Thr His Thr Gly
    290
                        295
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Ala Asp Gly Lys Ser Phe Arg Cys Val Gln Gln Gly Asp Asp Glu Asn
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                                        315
Gly Lys Ile Gly Val Ser Leu Ser Lys Asp Ile Thr Asp Val Ala Gly
                325
                                    330
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Arg Thr Val Lys Lys Asn Ile Ala Thr Leu Gly Pro Leu Ile Leu Pro
            340
                                345
Leu Ser Glu Lys Leu Leu Phe Phe Val Thr Phe Met Gly Lys Lys Leu
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                            360
Phe Lys Asp Lys Ile Lys His Tyr Tyr Val Pro Asp Phe Lys Leu Ala
                        375
                                            380
Ile Asp His Phe Cys Ile His Ala Gly Gly Arq Ala Val Ile Asp Glu
                    390
                                        395
Leu Glu Lys Asn Leu Gly Leu Ser Pro Ile Asp Val Glu Ala Ser Arg
                                    410
Ser Thr Leu His Arq Phe Glv Asn Thr Ser Ser Ser Ser Ile Trp Tyr
                                425
Glu Leu Ala Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Lys
                            440
Ala Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val
                        455
                                            460
Trp Val Ala Leu Arg Asn Val Lys Ala Ser Ala Asn Ser Pro Trp Gln
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                                        475
His Cys Ile Asp Arg Tyr Pro Val Lys Ile Asp Ser Asp Leu Ser Lys
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Ser Lys Thr His Val Gln Asn Gly Arg Ser
            500
<210> 33
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<211> 1524

<212> DNA <213> Artificial Sequence

<220>

<223> 1524 bp from B. napus elongase RCS (SEQ ID NO:3) having a mutation at position 920; designated Bn G307D; hypothetical

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ctt ttc aac ctt tgc ttc ttt ccg tta acg gcg atc gtc gcc gga aaa Leu Phe Asn Leu Cys Phe Phe Pro Leu Thr Ala Ile Val Ala Gly Lys 20 $$25\ $	96
gcc tat ogg ctt acc ata gac gat ctt cac cac tta tac tat tcc tat ala Tyr Arg Leu Thr Ile Asp Asp Leu His His Leu Tyr Tyr Ser Tyr 35 40 45	144
cto caa cac acc etc ata acc atc get cca ctc ttt gec ttc acc gtt Leu Gln His Asn Leu Ile Thr Ile Ala Pro Leu Phe Ala Phe Thr Val 50 $$	192
ttc ggt tcg gtt ctc tac atc gca acc cgg ccc aaa ccg gtt tac ctc Phe Gly Ser Val Leu Tyr Ile Ala Thr Arg Pro Lys Pro Val Tyr Leu 65 70 75 80	240
gtt gag tac toa tgc tac ctt coa coa acg cat tgt aga toa agt atc Val Glu Tyr Ser Cys Tyr Leu Pro Pro Thr His Cys Arg Ser Ser Ile 85 90 95	288
tco mag gto atg gat atc ttt tat cam gta aga aam get gat cct tct Ser Lys Val Met Asp Ile Phe Tyr Gin Val Arg Lys Ala Amp Pro Ser 100 100 105 110	336
cgg aac gge aeg tge gat gae teg teg tgg ett gae tte ttg agg aag arg ann clly ffr Cys asp asp Ser Ser Trp Leu Amp Phe Leu arg Lys 115 125 125 .	384
att caa gaa cgt toa ggt cta ggc gat gaa act cac ggg ccc gag ggg Ile Gln Glu Arg Ser Gly Leu Gly Asp Glu Thr His Gly Pro Glu Gly 135 140	432
ctg ctt cag gtc cct ccc cgg aag act ttt gog gog gog ogt gaa gag Leu Leu Gln Val Pro Pro Arg Lys Thr Phe Ala Ala Ala Arg Glu Glu 145 150 150 155	480
acg gag caa gtt atc att ggt gcg cta gaa aat cta ttc aag aac acc Thr Glu Gln Val Ile Ile Gly Ala Leu Glu Asn Leu Phe Lys Asn Thr 165 170 175	528
aac gtt aac cct aaa gat ata ggt ata ctt gtg gtg aac tca agc atg Asn Val Asn Pro Lys Asp Ile Gly Ile Leu Val Val Asn Ser Ser Met 180 185 190	576
ttt aat oca act oca tog oto too gog atg gto gtt aac act tto aag Phe Asn Pro Thr Pro Ser Leu Ser Ala Met Val Val Asn Thr Phe Lys 195 200 205	624
ctc cga agc aac gta aga agc ttt aac ctt ggt ggc atg ggt tgt agt Leu Arg Ser Asn Val Arg Ser Phe Asn Leu Gly Gly Met Gly Cys Ser 210 215 220	672
gcc ggc gtt ata gcc att gat cta gca aag gac ttg ttg cat gtc cat	720

Al 22		Gly	Val	Ile	Ala	Ile 230	Asp	Leu	Ala	Lys	Asp 235	Leu	Leu	His	Val	His 240	
									agc Ser								768
at II	t e	tac Tyr	gct Ala	ggt Gly 260	gat Asp	aat Asn	agg Arg	tcc Ser	atg Met 265	atg Met	gtt Val	tca Ser	aat Asn	tgc Cys 270	ttg Leu	ttc Phe	816
A	g	gtt Val	ggt Gly 275	ejà aaa	gcc Ala	gct Ala	att Ile	ttg Leu 280	ctc Leu	tcc Ser	aac Asn	aag Lys	cct Pro 285	gga Gly	gat Asp	egt Arg	864
	g								cac His								912
30 30	La	gac Asp	gac Asp	aag Lys	tct Ser	ttt Phe 310	cgt Arg	tgc Cys	gtg Val	caa Gln	caa Gln 315	gga Gly	gac Asp	gat Asp	gag Glu	aac Asn 320	960
G:	jc Ly	aaa Lys	atc Ile	gga Gly	gtg Val 325	agt Ser	ttg Leu	tcc Ser	aag Lys	gac Asp 330	ata Ile	acc Thr	gat Asp	gtt Val	gct Ala 335	ggt Gly	1008
									acg Thr 345								1055
L	a	agc Ser	gag Glu 355	aaa Lys	ctt Leu	ctt Leu	ttt Phe	ttc Phe 360	gtt Val	acc Thr	ttc Phe	atg Met	ggc Gly 365	aag Lys	aaa Lys	ctt Leu	1104
	1e								tac Tyr								1152
I.									gga Gly								1200
									ccg Pro								1248
									act Thr 425								1296
									gga Gly							aaa Lys	1344
									ggc				Asn				1392
t:	(D	gtg Val	gct Ala	cta Leu	aac Asn	aat Asn	gtc Val	aaa Lys	gct Ala	tcg Ser	aca Thr	aat Asn	agt. Ser	cct Pro	tgg Trp	gaa Glu	1440

465 470 475 480

cac tgc atc gac aga tac ccg gtc aaa att gat tct gat tca ggt aag
His Cys Ile Asp Arg Tyr Pro Val Lys Ile Asp Ser Asp Ser Gly Lys
485 485 490 495

tca gag act cgt gtc caa aac ggt cgg tcc taataa
Ser Glu Thr Arg Val Gln Asn Gly Arg Ser
505

<210> 34 <211> 506 <212> PRT

<213> Artificial Sequence

<220>

<223> 506 amino acids from B. napus elongase KCS (SEQ ID NO:4) having a mutation at residue 307; designated Bn G307D; hypothetical

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305					310	_		_		315		_			320	
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Arg	Thr	Val	Lys 340	Lys	Asn	Ile	Ala	Thr 345	Leu	Gly	Pro	Leu	Ile 350	Leu	Pro	
Leu	Ser	Glu 355	Lys	Leu	Leu	Phe	Phe 360	Val	Thr	Phe	Met	Gly 365	Lys	Lys	Leu	
Phe	Lys 370	Asp	Lys	Ile	Lys	His 375	Tyr	Tyr	Val	Pro	Asp 380	Phe	Lys	Leu	Ala	
11e 385	Asp	Hìs	Phe	Cys	Ile 390	His	Ala	Gly	Gly	Arg 395	Ala	Val	Ile	Asp	Val 400	
	Glu	ГЛЗ	Asn	Leu 405	Ala	Leu	Ala	Pro	Ile 410		Val	Glu	Ala	Ser 415		
Ser	Thr	Leu	His	Arg	₽he	Gly	Asn	Thr 425	Ser	Ser	Ser	Ser	Ile 430	Trp	Tyr	
Glu	Leu	Ala 435		Ile	Glu	Ala	Lys		Arg	Met	Lys	Lys 445		Asn	Lys	
Val	Trp		Ile	Ala	Leu	Gly 455		Gly	Phe	Lys	Cys 460		Ser	Ala	Val	
Trp		Ala	Leu	Asn	Asn 470		Lys	Ala	Ser	Thr 475		Ser	Pro	Trp	Glu 480	
	Cys	Ile	Asp	Arg	Tyr	Pro	Val	Lys	Ile 490		Ser	Asp	Ser	Gly 495		
Ser	Glu	Thr	Arg 500		Gln	Asn	Gly	Arg 505						-233		
					quen											
<221	3> 1' a hy L> CI	muta ypotl OS		rom I n at cal	A. tl pos:	hali								3		
<223 <223 <400	a hy 1: CI :> (: : : : : : : : : : : : : : : : : :	muta ypotl OS 1)	ation netion	rom In at cal	A. tl	halia ition	1 27	5; de	esign	nate	At	K921	R;		aac	48
<223 <221 <222 <400 atg	l> 1' a hy l> Cl > 3! acg	muta ypotl OS 1)	ation netion . (15:	rom In at cal	- A. tl	halia ition	a 27	ctt	tac	cgt	At tac	K921	R;	acc		48
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<223 <221 <222 <400 atg Met 1 ttt Phe	a hy l> Cl l> Cl l> Cl thr ttc Phe	muta ypoth OS 1) tcc ser aac Asn	gtt Val ctc Leu 20	rom In at cal 18) aac Asn 5 tgt Cys acc	gtt val	aag Lys ttc Phe	ctc Leu ccg Pro	ctt Leu tta Leu 25	tac Tyr 10 acg Thr	egt Arg geg Ala	tac Tyr ttc Phe	gtc Val ctc Leu	tta Leu gcc Ala 30	acc Thr 15 gga Gly	Asn aaa Lys ctc	
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<223 <222 <400 atg Met 1 ttt Phe gcc Ala caa Gln	l> 1 a hy l> Cl> Cl> Cl> Cl> Cl> Cl> Cl> Cl> Cl>	mutaypoth OS 1) tcc Ser aac Asn cgg Arg 35 aac Asn	gtt. Val ctc Leu ctt Leu ctt Leu	rom in at cal ls) aacc Asn 5 tgt Cys acc Thr ata Ile	gtt Val ttg Leu ata Ile	aag Lys ttc Phe aac Asn gta Val 55	ctc Leu ccg Pro gat Asp 40 act Thr	ctt Leu tta Leu 25 ctc Leu	tac Tyr 10 acg Thr cac His	cgt Arg gcg Ala aac Asn	tac Tyr ttc Phe ttc Phe ccg	gtc Val ctc Leu ctt Leu 45	tta Leu gcc Ala 30 tcc Ser act Thr	acc Thr 15 gga Gly tat Tyr	aaa Lys ctc Leu ttc Phe	96 144

Asp	Tyr	Ser	Суѕ	Tyr 85	Leu	Pro	Pro	Pro	His 90	Leu	Arg	Val	Ser	Val 95	Ser	
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					gat Asp											384
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					gag Glu											576
					tcg Ser											624
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					att Ile 230											720
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					aat Asn											816
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WO 01/94565 PCT/US01/18737

	325	330	335
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		cg atc gat gtg gag go ro Ile Asp Val Glu A 410	
	Arg Phe Gly Asn T	ct tca tct agc tca at hr Ser Ser Ser Ser I 25 4:	
		ga aga atg aag aaa g ly Arg Met Lys Lys G 445	
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		aa att gat tot gat to ys Ile Asp Ser Asp Lo 490	
	Val Gln Asn Gly A	gg toc taatttgatg ta rg Ser 05	totgagtg 1538
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Ala	ser	Arg 35		Thr	Ile	Asn	Asp 40		His	Asn	Phe	Leu 45		Tyr	Leu
Gln	His		Leu	Ile	Thr	Val 55		Leu	Leu	Phe	Ala		Thr	Val	Phe
Gly 65		Val	Leu	Tyr	Ile 70		Thr	Arg	Pro	Asn 75		Val	Tyr	Leu	Val 80
	Tyr	Ser	Cys			Pro	Pro	Pro		Leu	Arg	Val	Ser	Val	
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Arg	Asn	Val	100 Ala	Cys	Asp	Asp	Pro	105 Ser	Ser	Leu	Asp	Phe 125	110 Leu	Arg	Lys
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Leu		195 Ser	Asn	Ile	Lys		200 Phe	Asn	Leu	Gly		205 Met	Gly	Cys	Ser
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Arg	Val		260 Gly	Ala	Ala	Ile		265 Leu	Ser	Asn	Lys		270 Gly	Asp	Arg
Arg		275 Ser	Lys	Tyr	Lys		280 Val	His	Thr	Val		285 Thr	His	Thr	Gly
	290 Asp	qeA	ГЛЗ	Ser		295 Arg	Cys	Val	Gln	Gln	300 Glu	Asp	Asp	Glu	
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				405					410	Asp				415	
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		435					440			Met	-	445	_		
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Trp 465	Val	Ala	Leu	Arg	Asn 470	Val	Lys	Ala	ser	Ala 475	Asn	Ser	Pro	Trp	Gln 480
His	Cys	Ile	Asp	Arg	Tyr	Pro	Val	Lys	Ile	Asp	ser	Asp	Leu	ser	Lys

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gct gac gac aag Ala Asp Asp Lys 305		Cys Val Gln (
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cga acg gtt aag Arg Thr Val Lys 340				
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ttc aaa gat aaa Phe Lys Asp Lys 370				
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	ttg Leu															1344
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	tgc Cys														aag Lys	1488
	gag Glu									taa						1521
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Gly	Leu	Val	Leu	Tyr	Ile		Thr	Arg	Pro	Asn		Val	Tyr	Leu	Val	
65	Tyr	Car	Cara	Trees	70 Len	Dro	Pro	Dro	wie.	75 Ten	Tare	Va I	Car	1797	80	
-	•		-	85					90		-			95		
Ьyв	Val	Met	Asp 100	Ile	Phe	Tyr	Gln	Ile 105	Arg	Lys	Ala	Asp	Thr 110	Ser	Ser	
Arg	Asn	Val	Ala	Cys	Asp	Asp	Pro		Ser	Leu	Asp	Phe 125	Leu	Arg	Lys	

Lys Val Asn Pro Arg Glu Ile Gly Ile Leu Val Val Asn Ser Ser Met

150

Ile Glu Glu Arg Ser Gly Leu Gly Asp Glu Thr Tyr Ser Pro Glu Gly 130 135 140 Leu Ile His Val Pro Pro Arg Lys Thr Phe Ala Ala Ser Arg Glu Glu

Thr Glu Lys Val Ile Ile Gly Ala Leu Glu Asn Leu Phe Glu Asn Thr 165 170 175

220

Phe Asn Pro Thr Pro Ser Leu Ser Ala Met Val Val Asn Thr Phe Lys 200 Leu Arg Ser Asn Ile Lys Ser Phe Asn Leu Gly Gly Met Gly Cys Ser 215

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Gly Lys Ile Gly Val Ser Leu Ser Lys Asp Ile Thr Asp Val Ala Gly
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Ile Asp His Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Val
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Leu Glu Lys Asn Leu Ala Leu Ala Pro Ile Asp Val Glu Ala Ser Arg
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Ser Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr
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Glu Leu Ala Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Lys
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Val Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ala Val
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Trp Val Ala Leu Asn Asn Val Lys Ala Ser Thr Asn Ser Pro Trp Glu
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7
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	tct Ser															144
	cac His 50															192
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	att Ile															480
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	aat Asn															624
	cga Arg 210															672
	ggc															720
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att	tac	gct	ggt	gat	aat	agg	tac	atg	atg	gtt	tca	aat	tgc	ttg	ttc	816

Ile	туг	Ala	Gly 260	Asp	Asn	Arg	Ser	Met 265	Met	val	ser	Asn	Cys 270	Leu	Phe	
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								cac His								912
								gtg Val								960
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								ggc Gly								1392
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<212> PRT <213> Artificial Sequence

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365

Leu Ser Glu Lys Leu Leu Phe Phe Val Thr Phe Met Gly Lys Lys Leu

360

Phe Lys	Asp	Lys	Ile	Lys	His	Tyr	Tyr	Val	Pro	Asp 380	Phe	Lys	Leu	Ala	
Ile Asp	His	Phe	Cys	Ile	His	Ala	Gly	Gly	Arq	Ala	Val	Ile	Asp	Val	
385			•	390			•	•	395				-	400	
Leu Glu	Lys	Asn	Leu 405		Leu	Ala	Pro	Ile 410		Val	Glu	Ala	Ser 415		
Ser Thr	Leu			Phe	Gly	Asn			ser	Ser	Ser			Tyr	
		420				_	425	_		_	_	430	_	_	
Glu Leu	A1a 435	Tyr	Ile	Glu	Ala	Lys 440	Gly	Arg	Met	Lys	Lys 445	Gly	Asn	Lys	
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Trp Val	Ala	Leu	Asn	Asn 470	Val	гле	Ala	Ser	Thr 475	Asn	ser	Pro	Trp	Glu 480	
His Cys	TI.	7.00	71-		Dwo	Vr. 1	T ***	т1 о		Cox	7.00	cox	G7		
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Ser Glu	Thr	Arg 500	Val	Gln	Asn	Gly	Arg 505	Ser							
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	0:3)											Q ID			
M	0:1)	and	hav:	ing a	a mut	atio	on at	t nuc	cleot	ide	pos	ition	1		
	20;	desig	gnate	ed Br	1399	G30'	7D;]	nypot	:net:	LCal					
	20;	lesig	gnate	ed Br	1399	G30	7D; I	nypot	:het:	LCAI					
		lesig	gnate	ed Ba	1399	G30	7D; 1	nypot	:net:	LCAI					
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9: <221> Cl <222> (Cl <222>) (Cl <200> 400) 4' atg acg Met Thr 1 ctt ttc Leu Phe gcc tat Ala Tyr ctc caa Leu Gln 50 ttc ggt	DS 1) 1 tcc Ser aac Asn cgg Arg 35 cac	att Ile ctt Leu 20 ctt Leu aac Asn	aacc Cys acc Thr	gtt Val ttc Phe ata Ile ata	aag Lys ttt Phe gac Asp acc Thr 55	ctc Leu ccg Pro gat Asp 40 atc Ile	ctt Leu tta Leu 25 ctt Leu gct Ala	tac Tyr 10 acg Thr cac His	cat His gcg Ala cac His ctc Leu	tac Tyr atc Ile tta Leu ttt Phe 60	yal gtc Val tac Tyr 45 gcc Ala	gcc Ala 30 tat Tyr ttc Phe	Thr 15 gga Gly tcc ser acc Thr	Asn aaa Lys tat Tyr gtt Val	96 144
<pre>4221> Cl <222> () <400> 4: atg acg Met Thr 1 ctt ttc Leu Phe gcc tat Ala Tyr ctc caa Leu Gln 50 ttc ggt Phe Gly</pre>	DS 1) 1 tcc Ser aac Asn cgg Arg 35 cac	att Ile ctt Leu 20 ctt Leu aac Asn	aacc Cys acc Thr	gtt Val ttc Phe ata Ile ata	aag Lys ttt Phe gac Asp acc Thr 55	ctc Leu ccg Pro gat Asp 40 atc Ile	ctt Leu tta Leu 25 ctt Leu gct Ala	tac Tyr 10 acg Thr cac His	cat His gcg Ala cac His ctc Leu	tac Tyr atc Ile tta Leu ttt Phe 60	yal gtc Val tac Tyr 45 gcc Ala	gcc Ala 30 tat Tyr ttc Phe	Thr 15 gga Gly tcc ser acc Thr	Asn aaa Lys tat Tyr gtt Val ctc Leu	96 144 192
9: <221> Cl <222> (Cl <222>) (Cl <200> 400) 4' atg acg Met Thr 1 ctt ttc Leu Phe gcc tat Ala Tyr ctc caa Leu Gln 50 ttc ggt	DS 1) 1 tcc Ser aac Asn cgg Arg 35 cac	att Ile ctt Leu 20 ctt Leu aac Asn	aacc Cys acc Thr	gtt Val ttc Phe ata Ile ata Ile	aag Lys ttt Phe gac Asp acc Thr 55	ctc Leu ccg Pro gat Asp 40 atc Ile	ctt Leu tta Leu 25 ctt Leu gct Ala	tac Tyr 10 acg Thr cac His	cat His gcg Ala cac His ctc Leu	tac Tyr atc Ile tta Leu ttt Phe 60	yal gtc Val tac Tyr 45 gcc Ala	gcc Ala 30 tat Tyr ttc Phe	Thr 15 gga Gly tcc ser acc Thr	Asn aaa Lys tat Tyr gtt Val	96 144 192
<pre>4221> Cl <222> () <400> 4: add add add add add add add add add ad</pre>	DS 1) 1 tcc Ser aac Asn cgg Arg 35 cac His	att Ile ctt Leu 20 ctt Leu aac Asn	aac Asm 5 tgc Cys acc Thr ctc Leu ctc Leu	gtt Val ttc Phe ata Ile ata Ile tac Tyr 70	aag Lys ttt Phe gac Asp acc Thr 55 atc Ile	ctc Leu ccg Pro gat Asp 40 atc Ile	ctt Leu tta Leu 25 ctt Leu gct Ala acc	tac Tyr 10 acg Thr cac His	cat His gcg Ala cac His ctc Leu	tac Tyr atc Ile tta Leu ttt Phe 60 aaa Lys	yal gtc Val tac Tyr 45 gcc Ala ccg Pro	gcc Ala 30 tat Tyr ttc Phe	Thr 15 gga Gly tcc Ser acc Thr tac	aaa Lys tat Tyr gtt Val ctc Leu 80	96 144 192 240
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<pre>4221> Cl <222> () <400> 4: add add add add add add add add add ad</pre>	DS 1) 1 tcc Ser aacc Asn cgg Arg Arg Ser tcg tcg tcg tcg tcg tcg tcg tcg tcg tc	att Ile ctt Leu 20 ctt Leu aac Asn	aacc Asn 5 tgc Cys acc Thr ctc Leu tgc Cys	gtt Val ttc Phe ata Ile tac Tyr 70	aag Lys ttt Phe gac Asp acc Thr 55 atc Ile	ctc Leu ccg Pro gat Asp 40 atc Ile gca Ala	ctt Leu tta Leu 25 ctt Leu gct Ala acc Thr	tac Tyr 10 acg Thr cac His cca Pro	cat His gcg Ala cac His ctc Leu	tac Tyr atc Ile tta Leu ttt Phe 60 aaa Lys	Val gtc Val tac Tyr 45 gcc Ala ccg Pro	gcc Ala 30 tat Tyr ttc Phe gtt Val	Thr 15 gga Gly tcc Ser acc Thr tac Tyr agt Ser	Asn aaa Lys tat Tyr gtt Val ctc Leu 80 atc	96 144 192 240
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221) CC 422) CC 4400 AC 4 atg acg Met Thr 1 ctt ttc Leu Phe gcc tat Ala Tyr ctc caa Leu Gln 50 ttc ggt Phe Cly 65 gtt gag Val Glu	DS 1) 1 tcc Ser aac Asn cgg 35 cac His tcgr	att Ile ctt Leu 20 ctt Leu aacc Asn gtt Val	aac Asn 5 tgc Cys acc Thr ctc Leu ctc Leu tgc Cys 85	gtt Val ttc Phe ata Ile ata Tyr 70	aag Lys ttt Phe gac Asp acc Thr 55 atc Ile	ctc Leu ccg Pro gat Asp 40 atc Ile gca Ala cca	ctt Leu tta Leu 25 ctt Leu gct Ala acc Thr	tac Tyr 10 acg Thr cac His cca Pro	cat His gcg Ala cac His ctc Leu ccc Pro 75 cat	tac Tyr atc Ile tta Leu ttt Phe 60 aaa Lys	yal gtc Val tac Tyr 45 gcc Ala ccg Pro	gcc Ala 30 tat Tyr ttc Phe gtt Val tca Ser	Thr 15 gga Gly tcc Ser acc Thr tac Tyr agt Ser 95	aaa Lys tat Tyr gtt Val ctc Leu 80 atc Ile	96 144 192 240
s: <221> CC <222> (1) <420> 4) atg acg Met Thr 1 ctt ttc Leu Phe gcc tat ala Tyr ctc caa Leu Gln 50 ttc ggt Phe Gly 65 gtt gag Val Glu tcc aag	DS 1) 1 tcc Ser aac Asn cgg Arg 35 cac His tcg Ser tac Tyr	att Ile ctt Leu 20 ctt Leu aac Asn gtt Val tca ser	aacc Thr ctc Leu tgc Cys sgat	gtt Val ttc Phe ata Ile ata Ile tac Tyr 70 tac Tyr	aag Lys ttt Phe gac Asp acc Thr 55 atc Ile ctt Leu	ctc Leu ceg Pro gat Asp 40 atc Ile gca Ala cca	ctt Leu tta Leu 25 ctt Leu gct Ala acc Thr	tacc Tyr 10 aeg Thr cac His cca Pro	cat His geg Ala cac His ctc Leu ccc Pro 75 cat	tac Tyr atc Ile tta Leu ttt Phe 60 aaa Lys tgt Cys	Val gtc Val tac Tyr 45 gcc Ala ccg Pro	GCC Ala 30 tat Tyr ttc Phe gtt Val tca Ser gat	Thr 15 gga Gly tcc Ser acc Thr tac Tyr agt Ser 95	aaaa Lys tat Tyr gtt Val ctc Leu 80 atc Ile	96 144 192 240
221) CC 422) CC 4400 AC 4 atg acg Met Thr 1 ctt ttc Leu Phe gcc tat Ala Tyr ctc caa Leu Gln 50 ttc ggt Phe Cly 65 gtt gag Val Glu	DS 1) 1 tcc Ser aac Asn cgg Arg 35 cac His tcg Ser tac Tyr	att Ile ctt Leu 20 ctt Leu aac Asn gtt Val tca ser	aacc Thr ctc Leu tgc Cys sgat	gtt Val ttc Phe ata Ile ata Ile tac Tyr 70 tac Tyr	aag Lys ttt Phe gac Asp acc Thr 55 atc Ile ctt Leu	ctc Leu ceg Pro gat Asp 40 atc Ile gca Ala cca	ctt Leu tta Leu 25 ctt Leu gct Ala acc Thr	tacc Tyr 10 aeg Thr cac His cca Pro	cat His geg Ala cac His ctc Leu ccc Pro 75 cat	tac Tyr atc Ile tta Leu ttt Phe 60 aaa Lys tgt Cys	Val gtc Val tac Tyr 45 gcc Ala ccg Pro	GCC Ala 30 tat Tyr ttc Phe gtt Val tca Ser gat	Thr 15 gga Gly tcc Ser acc Thr tac Tyr agt Ser 95	aaaa Lys tat Tyr gtt Val ctc Leu 80 atc Ile	96 144 192 240

					gat Asp											384
					ggt Gly											432
					ccc Pro 150											480
					att Ile											528
					gat Asp											576
					tcg Ser											624
					aga Arg											672
					att Ile 230											720
					ctt Leu											768
					aat Asn											816
					gct Ala											864
					gag Glu											912
					ttt Phe 310											960
ggc Gly	aaa Lys	atc Ile	gga Gly	gtg Val 325	agt Ser	ttg Leu	tcc Ser	aag Lys	gac Asp 330	ata Ile	acc Thr	gat Asp	gtt Val	gct Ala 335	ggt Gly	1008
					aac Asn											1056

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	gc gag er Gli 355	Lys							1104
Phe L	aa gat ys Asg 70								1152
	ac cat sp His								1200
	ag aag lu Lys								1248
	og tta hr Lei								1296
	ta gca eu Ala 43:	Tyr							1344
Ala T	gg cag rp Glr 50								1392
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<213> Artificial Sequence

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<4005 42

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 Ser
 Ile
 Asn
 Val
 Lys
 Leu
 Tyr
 His
 Tyr
 Val
 Ile
 Thr
 Asn

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	Gly	Ser	Val	Leu		Ile	Ala	Thr	Arg		Lys	$_{\text{Pro}}$	Val	тух	
65	C17	m		~	70	*	Dece	n	m1	75	a	n			80
val	Glu			85					90					95	
Ser	Lys	Val	Met 100	Asp	Ile	Phe	Tyr	Gln 105	Val	Arg	Lys	Ala	Asp 110	Pro	ser
Arg	Asn	Gly 115	Thr	Cys	Asp	Asp	Ser	Ser	Trp	Leu	Asp	Phe 125	Leu	Arg	Lys
Ile	Gln 130	Glu	Arg	Ser	Gly	Leu 135	Gly	qaA	Glu	Thr	His 140	Gly	Pro	Glu	Gly
	Leu	G l n	Val	Pro			Lys	Thr	Phe			Ala	Arg	${\tt Glu}$	
145	~7				150			_		155	_		Ţ.,		160
	Glu			165		-			170				-	175	
Asn	Val	Asn	Pro 180	Lys	Asp	Ile	Gly	Ile 185	Leu	Val	Val	Asn	Ser 190	Ser	Met
Phe	Asn	Pro	Thr	Pro	Ser	Leu	Ser 200	Ala	Met	Val	Val	Asn 205	Thr	Phe	Lys
Leu	Arg 210	ser	Asn	Val	Arg	Ser 215	Phe	Asn	Leu	Gly	Gly 220	Met	Gly	Сув	Ser
Ala	Gly	Val	Ile	Ala	Ile		Leu	Ala	Lys	Asp		Leu	His	Val	His
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Ile	Tyr	Ala	Gly 260	Asp	Asn	Arg	Ser	Met 265	Met	Val	Ser	Asn	Cys 270	Leu	Phe
Arg	Val	Gly 275	Gly	Ala	Ala	Ile	Leu 280	Leu	Ser	Asn	ГЛS	Pro 285	Gly	Asp	Arg
Arg	Arg	Ser	Lys	Tyr	Glu	Leu	Val	His	Thr	Val	Arg	Thr	His	Thr	Gly
	290					295					300				
Ala	290 Asp	Asp	Lys	Ser	Phe					Gln	300				Asn
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305 Gly Arg Leu	Asp Lys Thr	Ile Val Glu 355	Gly Lys 340 Lys	Val 325 Lys Leu	310 Ser Asn Leu	Arg Leu Ile Phe	Cys Ser Ala Phe 360	Val Lys Thr 345 Val	Gln Asp 330 Leu Thr	315 Ile Gly Phe	300 Gly Thr Pro Met	Asp Asp Leu Gly 365	Asp Val Ile 350 Lys	Glu Ala 335 Leu Lys	320 Gly Pro Leu
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305 Gly Arg Leu Phe Ile 385	Asp Lys Thr Ser Lys 370 Asp	Ile Val Glu 355 Asp	Gly Lys 340 Lys Lys	Val 325 Lys Leu Ile Cys	310 Ser Asn Leu Lys Ile 390	Arg Leu Ile Phe His 375 His	Cys Ser Ala Phe 360 Tyr Ala	Val Lys Thr 345 Val Tyr	Gln Asp 330 Leu Thr Val	315 Ile Gly Phe Pro Arg 395	300 Gly Thr Pro Met Asp 380 Ala	Asp Leu Gly 365 Phe Val	Asp Val Ile 350 Lys Lys	Glu Ala 335 Leu Lys Leu Asp	320 Gly Pro Leu Ala Glu 400
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305 Gly Arg Leu Phe Ile 385 Leu	Asp Lys Thr Ser Lys 370 Asp	Ile Val Glu 355 Asp His	Gly Lys 340 Lys Lys Phe Asn	Val 325 Lys Leu Ile Cys Leu 405	310 Ser Asn Leu Lys Ile 390 Gly	Leu Ile Phe His 375 His Leu	Cys Ser Ala Phe 360 Tyr Ala Ser	Val Lys Thr 345 Val Tyr Gly	Gln Asp 330 Leu Thr Val Gly Ile 410	315 Ile Gly Phe Pro Arg 395 Asp	300 Gly Thr Pro Met Asp 380 Ala Val	Asp Asp Leu Gly 365 Phe Val Glu	Asp Val Ile 350 Lys Lys Ile Ala	Glu Ala 335 Leu Lys Leu Asp Ser 415	320 Gly Pro Leu Ala Glu 400 Arg
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305 Gly Arg Leu Phe Ile 385 Leu Ser Glu	Asp Lys Thr Ser Lys 370 Asp Glu Thr Leu	Ile Val Glu 355 Asp His Lys Leu Ala 435	Gly Lys 340 Lys Lys Phe Asn His 420 Tyr	Val 325 Lys Leu Ile Cys Leu 405 Arg	310 Ser Asn Leu Lys Ile 390 Gly Phe Glu	Arg Leu Ile Phe His 375 His Leu Gly Ala Gly	Cys Ser Ala Phe 360 Tyr Ala Ser Asn Lys 440	Val Lys Thr 345 Val Tyr Gly Pro Thr 425 Gly	Gln Asp 330 Leu Thr Val Gly Ile 410 Ser	Gly Phe Pro Arg 395 Asp Ser Met	300 Gly Thr Pro Met Asp 380 Ala Val Ser Lys Cys	Asp Leu Gly 365 Phe Val Glu Ser Lys 445	Asp Val Ile 350 Lys Lys Ile Ala Ile 430 Gly	Glu Ala 335 Leu Lys Leu Asp Ser 415 Trp Asn	320 Gly Pro Leu Ala Glu 400 Arg Tyr
305 Gly Arg Leu Phe Ile 385 Leu Ser Glu Ala Trp	Asp Lys Thr Ser Lys 370 Asp Glu Thr	Ile Val Glu 355 Asp His Lys Leu Ala 435 Gln	Gly Lys 340 Lys Lys Phe Asn His 420 Tyr	Val 325 Lys Leu Ile Cys Leu 405 Arg Ile Ala	310 Ser Asn Leu Lys Ile 390 Gly Phe Glu Leu Asn	Arg Leu Ile Phe His 375 His Leu Gly Ala Gly 455	Cys Ser Ala Phe 360 Tyr Ala Ser Asn Lys 440 Ser	Val Lys Thr 345 Val Tyr Gly Pro Thr 425 Gly Gly	Gln Asp 330 Leu Thr Val Gly Ile 410 Ser Arg	315 Ile Gly Phe Pro Arg 395 Asp Ser Met Lys	300 Gly Thr Pro Met Asp 380 Ala Val Ser Lys Cys 460	Asp Leu Gly 365 Phe Val Glu Ser Lys 445 Asn	Asp Val Ile 350 Lys Lys Ile Ala Ile 430 Gly Ser	Glu Ala 335 Leu Lys Leu Asp Ser 415 Trp Asn	320 Gly Pro Leu Ala Glu 400 Arg Tyr Lys Val
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305 Gly Arg Leu Phe Ile 385 Leu Ser Glu Ala Trp 465 His	Asp Lys Thr Ser Lys 370 Asp Glu Thr Leu Trp 450 Val	Ile Val Glu 355 Asp His Lys Leu Ala 435 Gln Ala	Gly Lys 340 Lys Lys Phe Asn His 420 Tyr Ile Leu Asp	Val 325 Lys Leu Ile Cys Leu 405 Arg Ile Ala Arg	310 Ser Asn Leu Lys Ile 390 Gly Phe Glu Leu Asn 470 Tyr	Arg Leu Ile Phe His 375 His Leu Gly Ala Gly 455 Val	Cys Ser Ala Phe 360 Tyr Ala Ser Asn Lys 440 Ser Lys Val	Val Lys Thr 345 Val Tyr Gly Pro Thr 425 Gly Gly Ala Lys	Gln Asp 330 Leu Thr Val Gly Ile 410 Ser Arg Phe Ser Ile 490	315 Ile Gly Phe Pro Arg 395 Asp Ser Met Lys Ala 475	300 Gly Thr Pro Met Asp 380 Ala Val Ser Lys Cys 460 Asn	Asp Leu Gly 365 Phe Val Glu Ser Lys 445 Asn Ser	Asp Val Ile 350 Lys Lys Ile 430 Gly Ser Pro	Glu Ala 335 Leu Lys Leu Asp Ser 415 Trp Asn Ala Trp Ser	320 Gly Pro Leu Ala Glu 400 Arg Tyr Lys Val Gln 480

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<210> 54 <211> 31 <212> DNA

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WHAT IS CLAIMED IS:

A polypeptide comprising in the amino-terminal to carboxy-terminal direction:

- a first polypeptide segment, wherein said first polypeptide segment has membrane anchoring properties; joined to
 - (b) a second polypeptide segment having a sequence selected from the group consisting of residues 75-114 of SEQ ID NO:12 and residues 75-114 of SEQ ID NO:14; joined to
 - (c) a third polypeptide segment having at least 40% sequence identity to residues 115-506 of SEO ID NO:4.
 - The polypeptide of claim 1, wherein said third polypeptide segment has at least 50% sequence identity to residues 115-506 of SEQ ID NO:4.

The polypeptide of claim 2, wherein said third polypeptide segment has an
aspartic acid at the position corresponding to amino acid 307 of SEQ ID NO.4.

- $\begin{tabular}{ll} 4. & The polypeptide of claim 3, wherein said polypeptide has the amino acid \\ 20 & sequence of SEQ ID NO: 20. \end{tabular}$
 - 5. The polypeptide of claim 3, wherein said polypeptide has the amino acid sequence of SEQ ID NO:22.
- The polypeptide of claim 3, wherein said polypeptide has the amino acid sequence of SEO ID NO:34.
 - 7. The polypeptide of claim 3, wherein said polypeptide has the amino acid sequence of SEQ ID NO:36.

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 The polypeptide of claim 1, wherein said polypeptide catalyzes the condensation of malonyl CoA and a C18 fatty acyl substrate, leading to the synthesis of a C20 fatty acyl CoA.

- The polypeptide of claim 8, wherein said C18 fatty acyl substrate is an oleovl substrate.
- The polypeptide of claim 1, wherein said polypeptide catalyzes the condensation of malonyl CoA and a C20 fatty acyl substrate, leading to the synthesis of a
 C22 fatty acyl CoA.
 - The polypeptide of claim 10, wherein said C20 fatty acyl substrate is an eicosenoyl substrate.
- 15 12. A nucleic acid encoding the polypeptide of claim 1.
 - 13. A nucleic acid encoding the polypeptide of claim 2.
 - 14. A nucleic acid encoding the polypeptide of claim 3.
 - 15. Host cells containing a nucleic acid encoding the polypeptide of claim 1.
 - 16. Host cells containing a nucleic acid encoding the polypeptide of claim 2.
- Host cells containing a nucleic acid encoding the polypeptide of claim 3.
 - 18. The host cells of claim 15, wherein said host cells are yeast cells.
 - 19. The host cells of claim 15, wherein said host cells are plant cells.

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	20.	A plant containing an exogenous nucleic acid encoding the polypeptide of
	claim 1.	
5	21. claim 2.	A plant containing an exogenous nucleic acid encoding the polypeptide of
	22. claim 3.	A plant containing an exogenous nucleic acid encoding the polypeptide of
10	23.	The plant of claim 20, wherein said plant is Brassica napus.
	24.	The plant of claim 21, wherein said plant is Brassica napus.
15	25.	The plant of claim 22, wherein said plant is Brassica napus.

Arabidopsis thaliana FAE1 (SEQ ID NO:2)

MTSVNVKLLY RYVLTNEFNI CLFPLTAFLA GKASRLITID LHNFLSYLÇH
NLITVILLFA FTVFGLVLYI VTRPNFVYLV DYSCYLPPPH LKVSVSKVMD
IFYQIRKADT SSRNVACDDP SSLDFLRKIQ ERSGLGDETY SPEGLIHVPP
RTTFAASREE TEKVIIGALE NLFENTKVNP REIGILVVNS SMENPIPSLS
AMVUNFFKLK SNIKSPHIGG MGCSAGVIAI DLAKDLLHVH KNYTALVVST
ENITGGIYAG ENRSMMVSNC LFRVGGAAIL LSNKSGDRRR SKYKLVHTVR
THTGADDKSF RCVQQEDDES GKIGVCLSKD ITNVAGTILT KNIATLGPLI
LPLSEKFLFF AFFVAKKLUK DKIKHYVPDF FKLAUDHFCI HAGGRAVIDS
LEKNLGLSPI DVEASRSTLH RFGNTSSSSI WYELAYIEAK GRMKKGNKAW
QIALGSGFKC NSAVWVALRN VKASANSPWQ HCIDRYPVKI DSDLSKSKTH
VQNGRS

Brassica napus elongase KCS (SEQ ID NO:4)

MTSINVKLLY HYVITNLFNL CFFPLTAIVA GRAYRLTIDD LHRLYYSYLQ
HNLITIAPLF AFTVFGSVLY IATRPKPVYL VEYSCYLPPT HCRSSISKUM
DIFYQVRRAD PSRNGTODDS SWLDFILKKIQ ERSGLGDETH GEBGLLQVFP
RXTFRAARRE TEQVIIGALE NLFKNTNVNP KDIGILVVNS SMENPTPSLS
AMVVNTFKLR SNVRSFNLGG MGCSAGVIAI DLAKDLLHVH KNTYALVVST
ENITNNIYAG DNRSMMYSKC LFRVGGAAIL LSNKEGDRRR SKYELVHTVR
THTGADGKSF RCVQQGDDEN GKIGVSLSKD ITDVAGRTVK KNIATLGFLI
LPLSEKLLFF VTFMGKLEK DKIKHYVYPD FKLAIDHFCI HAGGRAVIDV
LEKNLALAPI DVEASRSTLH RFGNTSSSSI WYELAYIEAK GRMKKGNKVW
QIALGSGFKC NSAVWVALNN VKASTNSPWE HCIDRYPVKI DSDSGKSETR
VQNGRS

B. napus elongase KCS (SEQ ID NO:6)

MTSINVALLY HYVINLENL CFFPLTAIVA GKAYLTIDDI HHLYYSYLOH
NLITIAPILA FTVFGSVLYI ATRPKPYYLV EYSCYLPPTH CRSSISKYMD
IFFQVKKADP SRNGTCDDSS WLDFIRKIQE RSCLGDETHG PEGLLQVPPR
KTFRARREET EQVIIGALEN LEKNTNVAPK DIGILVYNSS MFNPTFSISA
MVVNTFKLRS NVRSFNLGGM GCSAGVIAID LAKDLHVHK NTYALVVSTE
NITYNIYAGD NRSMYNSNCL FRVGGAALLL SKKPRDRRSS KYELHTYRI
HTGADDKSFF CVQQGDDENG GTGVSLSKDI TDVAGRTVKK NIATLEPLIL
PLSEKLLFFV TFMGKKLFKD EIKHYYPDF KLAIDHFCH AGGKAVIDVL
EKNLGLAPID VEASRSTLHR FGNTSSSSIW YELAYIEPKG RMKKGNKVWQ
LALGSGFKCN SAVWVALNNV KASTNSPWEH CIDRYPVKID SDEGKSETRV

FIGURE 1-1

At114 (SEQ ID NO:8)

MTSVNVKLLY RYVLTNFFNI CLFPLTAFIA GKASKLITID LHNFLSYLQH
NLITVTLLFA FTVFGLVLYI VTRPNFVYLV DYSCYLPPPH LKVSVSKVMD
LFYQIRKADT SSRNGTCDDS SWLDFIRKIQ BERGLGOETH GFEGLLQVPP
RKTFAAARRE TEQVIIGALE NLFKNTNVNP KDIGILVVNS SMFNPTPSLS
AMVVNTFKLR SNVRSFRLG MGCSAGVIAI DLAKDLLHVH KNTYALVVST
ENITYNIYAG DNRSMMYSNC LFRVGGAALL LSNKFGDRRR SKYELVHTVR
THTGADGKSF RCVQQGDDEN GKIGVSLSKD ITDVAGGRYVK KNIATLGFLI
LPLSEKLLFF VTFMGKKLFK DKIKHYVVPD FKLAIDHFCI HAGGRAVIDV
LEKNLALAPI DVEASRSTLH RFGNTSSSSI WYELAYIEAK GRMKKGNKVW
QIALGSGFKC NSAVWVALNN VKASTNSPWE HCIDRYPVKI DSDSGKSETR

At74 (SEQ ID NO:10)

MISVNVKLLY RYVLTNEFNI CLFPLTAFLA GKASRLITND LHNFLSYLQH
NLITVILFA FTVFGLVLYI VTRPKPVYLV EYSCYLPPTH CRSSISKVMD
IFYQVRKADP SRNGTCDDSS WLDFLRRIQE REGLEDETHE PEGLLQVPPR
KFFAAAREET EQVIIGALEN LFKNTNVNPK DIGLLVVNSS MFPPTFSLSA
MVVNTFKLES NVRSFNLGGM GCSAGVIAID LAKDLLHVHK NTYALVVSTE
NITYNIYAGD NRSMMYSNCL FRVGGAALL SNKPGDRRS KYELVHTVRI
HTGADGKSFR CVQGDDENG KIGVSLSKDI TDVAGRTVKK NIATLEPILL
PLSEKLLFFV TFMKKKLFED KIKHYVPDF KLAIPHFCH AGGRAVIOVL
EKNLALAPID VEASRSTLHR FGNTSSSIW YELAYIEAKG RMKKGNKVWQ
IALGSGFKCN SAVWVALNNV KASTNSPWEH CIDRYPVKID SDSGKSETRV

At114 L91C K92R (SEO ID NO:12)

MISVNYKLLY RYVLTNEFNI CLEPLITAFLA GKASRLITID LHNFLSYLOH
NLITYTLLFA FTVFGLVLYI VTRPNFVYLV DYSCYLPFPH CRVSVSKYMD
IFYQIRKADT SSRNGTODNS SWLDFLRKIQ ERSGLGOETH GEBGLLQVFP
RKTFAAARRE TEQVIIGALE NLFKNTNVNP KDIGILVVNS SMFNPTFSLS
AMVVNTFKLR SNVRSFNLGG MGCSAGVIAI DLAKDLLHVH KNTYALVVST
ENITYNIYAG DNRSMMYSKC LFRVGGAAIL LENKFGDRRR SKXELVHTVR
THTGADGKSF RCVQQGDDEN GKIGVSLSKD ITDVAGRTVK KNIATLGFLI
LELSEKLLFF VTFMGKALEK DKIKHYVPUP FKLALDHFCI HAGGRAVIDV
LEKNLALAPI DVEASRSTLH RFGNTSSSSI WYELAYLEAK GRMKKGNKVW
VRGRS

UNAUSWALNN VKASTNSPWE HCIDRYPVKI DSDSGKSETR
VPNGRS

FIGURE 1-2

At114 K92R (SEQ ID NO:14)

MTSVNVKLLY	RYVLTNFFNL	CLFPLTAFLA	GKASRLTIND	LHNFLSYLQH
NLITVTLLFA	FTVFGLVLYI	VTRPNPVYLV	DYSCYLPPPH	LRVSVSKVMD
IFYQIRKADT	SSRNGTCDDS	SWLDFLRKIQ	ERSGLGDETH	GPEGLLQVPP
RKTFAAAREE	TEQVIIGALE	NLFKNTNVNP	KDIGILVVNS	SMFNPTPSLS
AMVVNTFKLR	SNVRSFNLGG	MGCSAGVIAI	DLAKDLLHVH	KNTYALVVST
ENITYNIYAG	DNRSMMVSNC	LFRVGGAAIL	LSNKPGDRRR	SKYELVHTVR
THTGADGKSF	RCVQQGDDEN	GKIGVSLSKD	ITDVAGRTVK	KNIATLGPLI
LPLSEKLLFF	VTFMGKKLFK	DKIKHYYVPD	FKLAIDHFCI	HAGGRAVIDV
LEKNLALAPI	DVEASRSTLH	RFGNTSSSSI	WYELAYIEAK	GRMKKGNKVW
QIALGSGFKC	NSAVWVALNN	VKASTNSPWE	HCIDRYPVKI	DSDSGKSETR
VPNGRS				

At114 G307D (SEQ ID NO:16)

MTSVNVKLLY	RYVLTNFFNL	CLFPLTAFLA	GKASRLTIND	LHNFLSYLQH
NLITVTLLFA	FTVFGLVLYI	VTRPNPVYLV	DYSCYLPPPH	LKVSVSKVMD
IFYQIRKADT	SSRNGTCDDS	SWLDFLRKIQ	ERSGLGDETH	GPEGLLQVPP
RKTFAAAREE	TEQVIIGALE	NLFKNTNVNP	KDIGILVVNS	SMFNPTPSLS
AMVVNTFKLR	SNVRSFNLGG	MGCSAGVIAI	DLAKDLLHVH	KNTYALVVST
ENITYNIYAG	DNRSMMVSNC	LFRVGGAAIL	LSNKPGDRRR	SKYELVHTVR
THTGADDKSF	RCVQQGDDEN	GKIGVSLSKD	ITDVAGRTVK	KNIATLGPLI
LPLSEKLLFF	VTFMGKKLFK	DKIKHYYVPD	FKLAIDHFCI	HAGGRAVIDV
LEKNLALAPI	DVEASRSTLH	RFGNTSSSSI	WYELAYIEAK	GRMKKGNKVW
QIALGSGFKC	NSAVWVALNN	VKASTNSPWE	HCIDRYPVKI	DSDSGKSETR
VQNGRS				

At74 G306D (SEQ ID NO:18)

MTSVNVKLLY	RYVLTNFFNL	CLFPLTAFLA	GKASRLTIND	LHNFLSYLQH
NLITVTLLFA	FTVFGLVLYI	VTRPKPVYLV	EYSCYLPPTH	CRSSISKVMD
IFYQVRKADP	SRNGTCDDSS	WLDFLRKIQE	RSGLGDETHG	PEGLLQVPPR
KTFAAAREET	EQVIIGALEN	LFKNTNVNPK	DIGILVVNSS	MFNPTPSLSA
MVVNTFKLRS	NVRSFNLGGM	GCSAGVIAID	LAKDLLHVHK	NTYALVVSTE
NITYNIYAGD	NRSMMVSNCL	FRVGGAAILL	SNKPGDRRRS	KYELVHTVRT
HTGADDKSFR	CVQQGDDENG	KIGVSLSKDI	TDVAGRTVKK	NIATLGPLIL
PLSEKLLFFV	TFMGKKLFKD	KIKHYYVPDF	KLAIDHFCIH	AGGRAVIDVL
EKNLALAPID	VEASRSTLHR	FGNTSSSSIW	YELAYIEAKG	RMKKGNKVWQ
IALGSGFKCN	SAVWVALNNV	KASTNSPWEH	CIDRYPVKID	SDSGKSETRV
QNGRS				

FIGURE 1-3

At114 L91C K92R G307D (SEO ID NO:20)

MISVNVKLLY RYVLINFFNL CLFPLTAFLA GRASRLITND LHNFLSYLQH
NLITVILFA FTVFGLVLYL VTRPNPVYLV DYSCYLPPPH CRVVSKVMD
IFYQIRKADT SSRNGTCDNS SWLDFILKKIQ ERSGLGDETH GEGGLLQVPP
RKTFAAAREE TEQVIIGALE NLFKNTNVNP KDIGILVVNS SMENPTPSLS
AMVVNTFKLR SNVRSFNLGG MGCSAGVIAI DLAKDLHIVH KNTYALVVST
ENITYNIYAG DNRSMNVSNC LFRVGGAALL LENKEGDRRR SKYELVHTVR
THTGADDKSF RCVQOGDDEN GKIGVSLSKD ITDVAGRTVK KNIATIGFLI
LPLSEKLIFF VTFMGKLIFK DKIKHYVVPD FKLAIDHFCI HAGGRAVIDV
LEKNLALAPI DVEASRSTLH RFGRISSSSI WYELAYIEAK GRMKKGNKVW
QIALGSGFKC NSAVWVALNN VKASTNSPWE HCIDRYPVKI DSDSGKSETR
VONGRS

At114 K92R G307D (SEQ ID NO:22)

MTSVNVKLLY RYULTNEFNI CLEPLTAFLA GKASRLITIND LHNFLSYLCH
NLITVTLIFA FTVFGLVLYI VTRPNFVYLV DYSCYLPPPH LRVSVSKVMD
IFYQIRKADT SSRNGTCDDS SWLDFILKRIQ ERSGLGDETH GPEGLLQVPP
RKIFAAAREE TEQVICALE NIFKNTNNIP KDIGILVVNS SMFNPTPSLS
AMVNNFKLR SNVRSFRLGG MCSAGVIAI DLAKDLLHVH KNTYALVVST
ENITYNIYAG DNSSMVSNC LFRVGGAAIL LSNKFGDRRR SXYELVHTVR
THTGADDKSF RCVQQGDDEN GKIGVSLSKD ITDVAGRTVK KNIATIGELI
LEJSEKLIFF VTFMGKLFK DKIKHYVPUP FKLAIDHFCI HAGGRAVIDV
LEKNLALAPI DVEASRSTLH RFGNTSSSSI WYELAYIEAK GRMKKCNKVW
QIALGSGFKC NSAVWVALNN VKASTNSPWE HCIDRYPVKI DSDSGKSETR
VONGRS

At254 (SEQ ID NO:24)

MTSVNVKLLY RYVLTNFFNL CLFPLTAFLA GKASRLITID LHNLLSYLOH
NLITVTLLFA FTVFGLVLYI VTRPNPVYLV DYSCYLPPPH LKVSVSKVMD
IFYQIRKADT SSRNVACDDP SSLDFIRKIQ ERSGLGDETY SFEGLIHVPP
RKTFAASREE TEKVIIGALE NLFENTKVNF REIGILVVNS SMENPFPSLS
RMTVNTFKLR SNIKSFNLGG MGCSAGVIAI DLAKDLHVH KNTYALVVST
ENITYNIYAG DNRSMMVSNC LFRVGGAAIL LSNKFGDRRR SKYELVHTVR
THTGADGKSF RCVQCGDDEN GKIGVSLSKD ITDVAGRTVK KNIATLGFLI
LPLSEKLIFF VTFMGKKLFK DKIKHYVVPD FKLAIDHFCI HAGGRAVIDV
LEKNLALAPI DVEASRSTLH RFGTTSSSSI WYELAYIEAK GRMKKGNKVW
QIALGSGFKC NSAVWVALNN VKASTNSPWE HCIDRYPVKI DSDSGKSETR

FIGURE 1-4

At173 (SEO ID NO:26)

MTSVNVKLLY	RYVLTNFFNL	CLFPLTAFLA	GKASRLTIND	LHNFLSYLQH
NLITVTLLFA	FTVFGLVLYI	VTRPNPVYLV	DYSCYLPPPH	LKVSVSKVMD
IFYQIRKADT	SSRNVACDDP	SSLDFLRKIQ	ERSGLGDETY	SPEGLIHVPP
RKTFAASREE	TEKVIIGALE	NLFKNTNVNP	KDIGILVVNS	SMFNPTPSLS
AMVVNTFKLR	SNVRSFNLGG	MGCSAGVIAI	DLAKDLLHVH	KNTYALVVST
ENITYNIYAG	DNRSMMVSNC	LFRVGGAAIL	LSNKPGDRRR	SKYELVHTVR
THTGADGKSF	RCVQQGDDEN	GKIGVSLSKD	ITDVAGRTVK	KNIATLGPLI
LPLSEKLLFF	VTFMGKKLFK	DKIKHYYVPD	FKLAIDHFCI	HAGGRAVIDV
LEKNLALAPI	DVEASRSTLH	RFGNTSSSSI	WYELAYIEAK	GRMKKGNKVW
QIALGSGFKC	NSAVWVALNN	VKASTNSPWE	HCIDRYPVKI	DSDSGKSETR
VQNGRS				

Bn176 (SEO ID NO:28)

MTSINVKLLY HYVITNLFNL CFFPLTAIVA GRAYRLITIDD LHHLYYSYIQ

DIFYQVRKAD PSRNGTCDDS SWLDFILKRIQ ERSGLGDETH GPEGLLQVPF

RKFFAAAREE TEQVIIGALE NLFKNTKVNP REIGILVVNS SMFNPFPSLS

RMVNNFFKLR SNIKSFNLG MGCSACVIAI DLAKDLLHVH KNTYALVVST

ENITQGIYAG ENRSMWYSNC LFRVGGAAIL LSNKSGDRRR SKYKLVFTVR

THTGADDKSF RCVQQEDDES GKIGVCLSKD INVAGTTLT KNIATIGFLI

LEXSEKPLEF ATFVAKKLK DKIKHYVPDF FKLAUDHFCI HAGGRAVIDE

LEKNLGLSPI DVEASRSTLH RFGNTSSSSI WYELAYIEAK GRMKKCNKAW

QIALGSGFKC NSAVWVALRN VKASANSPWQ HCIDRYPVKI DSDLSKSKTH

VONGRS

At399 (SEO ID NO:30)

MTSVNVKLLY	RYVLTNFFNL	CLFPLTAFLA	GKASRLTIND	LHNFLSYLQH
NLITVTLLFA	FTVFGLVLYI	VTRPNPVYLV	DYSCYLPPPH	LKVSVSKVMD
IFYQIRKADT	SSRNVACDDP	SSLDFLRKIQ	ERSGLGDETY	SPEGLIHVPP
RKTFAASREE	TEKVIIGALE	NLFENTKVNP	REIGILVVNS	SMFNPTPSLS
AMVVNTFKLR	SNIKSFNLGG	MGCSAGVIAI	DLAKDLLHVH	KNTYALVVST
ENITQGIYAG	ENRSMMVSNC	LFRVGGAAIL	LSNKSGDRRR	SKYKLVHTVR
THTGADDKSF	RCVQQEDDES	GKIGVCLSKD	ITNVAGTTLT	KNIATLGPLI
LPLSEKFLFF	ATFVAKKLLK	DKIKHYYVPD	FKLAVDHFCI	HAGGRAVIDV
LEKNLALAPI	DVEASRSTLH	RFGNTSSSSI	WYELAYIEAK	GRMKKGNKVW
QIALGSGFKC	NSAVWVALNN	VKASTNSPWE	HCIDRYPVKI	DSDSGKSETR
VQNGRS				

FIGURE 1-5

Bn399 (SEO ID NO:32)

MTSINVKLLY HYVITNIFUL OFFPLTAIVA GKAYRLITID LHHLYYSYLQ
HNLITIAPLF AFTVFGSVLY LATRPKPVYL VEYSCYLPPT HCRSSISKVM
DIFYQVRKAD PSRNGTCDDS SWLDFILKKIQ ERSGLGDETH GFBGLLQVPF
RKTFAAARFE TEQVIIGALE NIFKNTNVNP KDIGILVVNS SMENPTPSLS
AMVWNFFKLR SNVRSFNLGG MGCSAGVIAI DLAKDLLHVH KNTYALVVST
ENITYNIYAG DNRSMMYSNC LFRVGGAALL LSKNFGBRRR SKYEUVHTVR
THTGADGKSF RCVQQGDDEN GKIGVSLSKD ITDVAGGTVVK KNIATIGELI
LEKNLGISFI VFFMGKKLEK DKIKHYVPUP FKLAIDHECI HAGGRAVIDE
LEKNLGISFI DVEASRSTLH RFGNTSSSSI WYELAYIEAK GRMKKGNKAW
QIALGSGFKC NSAVWVALRN VKASANSPWQ HCIDRYFVKI DSDLSKSKTH
VQNGRS

Bn G307D (SEO ID NO:34)

MTSINVKLLY HYVITNLFNI CFFPLTAIVA GKAYRLTIDD LHHLYYSYLQ
HNLITIAPLF AFTVFGSVLY IATREKPVYL VEYSCYLPPT HCRSSISKUM
DIFYQVRKAD PSRNGTCDDS SWLDFIRKIQ ERSGLGDETH GPEGLLQVFP
RKWFRAARREE TEQVIIGALE NLFKNTNVNP KDIGILVVNS SMENPPPSLS
AMVVNTFKLR SNVRSFNLGG MGCSAGVIAI DLAKDLLHVH KNTYALVVST
ENITYNIYAG DNRSMMYSNC LFRVGGAAIL LSNKFGDRRR SKYELVHTVR
THTGADDKSF RCVQQGDDEN GKIGVSLSKD ITDVAGRTVK KNIATLGFLI
LPLSEKLLFF VTFMGKLKFK DKIKHYVYPD FKLAIDHFCI HAGGRAVIDV
LEKNLALAPI DVEASRSTLH RFGNTSSSSI WYELAYIEAK GRNKKGNKVW
QIALGSGFKC NSAVWVALNN VKASTNSPWE HCIDRYPVKI DSDSGKSETR
VONGERS

At K92R (SEO ID NO:36)

MISYNVKLLY RYVLTNIFNI CLFPLTAFLA GKASRLITID LHNFLSYLOR
NLITVILFA FTVFGLVLYI VTRPNFVYLV DYSCYLPPPH LRVSVSKVMD
LFYQIRKADT SSRNVACDDP SSLDFLKKIQ ERSGLGDETY SPEGLIHVPP
RKTFAASREE TEKVIIGALE NLFENTKVNP REIGILVVNS SMFNPTPSLS
AMVUNFFKLR SNIKSFNLGG MGCSAGVIAI DLAKDLHVH KNTYALVVST
ENITQGITAG ENRSMMYSNC LFRVGGAAIL LENKSGDRRR SKYKLVHTVR
THIGADDKSF RCVQQEDDES GKIGVCLSKD ITNVAGTTLT KNIATLGPLI
LEKSKELFE ATFVAKKLK DKIKHYVPD FKLAVDHFCI HAGGRAVIDE
LEKNLGLSPI DVEASRSTLH RFGNTSSSSI WYELAYLEAK GRMKKGNKAW
QIALGSGFKC NSAVWVALRN VKASANSPWQ HCIDRYPVKI DSDLSKSKTH
VONGRS

FIGURE 1-6

At254 G307D (SEO ID NO:38)

MTSVNVKLLY	RYVLTNFFNL	CLFPLTAFLA	GKASRLTIND	LHNFLSYLQH
NLITVTLLFA	FTVFGLVLYI	VTRPNPVYLV	DYSCYLPPPH	LKVSVSKVMD
IFYQIRKADT	SSRNVACDDP	SSLDFLRKIQ	ERSGLGDETY	SPEGLIHVPP
RKTFAASREE	TEKVIIGALE	NLFENTKVNP	REIGILVVNS	SMFNPTPSLS
AMVVNTFKLR	SNIKSFNLGG	MGCSAGVIAI	DLAKDLLHVH	KNTYALVVST
ENITYNIYAG	DNRSMMVSNC	LFRVGGAAIL	LSNKPGDRRR	SKYELVHTVR
THTGADDKSF	RCVQQGDDEN	GKIGVSLSKD	ITDVAGRTVK	KNIATLGPLI
LPLSEKLLFF	VTFMGKKLFK	DKIKHYYVPD	FKLAIDHFCI	HAGGRAVIDV
LEKNLALAPI	DVEASRSTLH	RFGNTSSSSI	WYELAYIEAK	GRMKKGNKVW
QIALGSGFKC	NSAVWVALNN	VKASTNSPWE	HCIDRYPVKI	DSDSGKSETR
VQNGRS				

At173 G307D (SEQ ID NO:40)

MISVNVKLLY	RYVLTNFFNL	CLFPLTAFLA	GKASRLTIND	LHNFLSYLQH
NLITVTLLFA	FTVFGLVLYI	VTRPNPVYLV	DYSCYLPPPH	LKVSVSKVMD
			ERSGLGDETY	
RKTFAASREE	TEKVIIGALE	NLFKNTNVNP	KDIGILVVNS	SMFNPTPSLS
AMVVNTFKLR	SNVRSFNLGG	MGCSAGVIAI	DLAKDLLHVH	KNTYALVVST
ENITYNIYAG	DNRSMMVSNC	LFRVGGAAIL	LSNKPGDRRR	SKYELVHTVR
THTGADDKSF	RCVQQGDDEN	GKIGVSLSKD	ITDVAGRTVK	KNIATLGPLI
LPLSEKLLFF	VTFMGKKLFK	DKIKHYYVPD	FKLAIDHFCI	HAGGRAVIDV
LEKNLALAPI	DVEASRSTLH	RFGNTSSSSI	WYELAYIEAK	GRMKKGNKVW
QIALGSGFKC	NSAVWVALNN	VKASTNSPWE	HCIDRYPVKI	DSDSGKSETR
VQNGRS				

Bn399 G307D (SEQ ID NO:42)

MTSINVKLLY HYUITNLFNL CFFPLTAIVA GRAYRLITIOD LHRLYYSYLQ
HNLITIAPLF AFTVFGSVLY IATRFKPLYL VEYSCYLPPT HCRSSISKUM
DIFYQVRKAD PSRNGTCDDS SWLDFLRKIQ BRSGLGDETH GFBGLLQVFP
RKTFRAARRE TEQVIIGALE NLFKNTNNTP KDIGILVVNS SMFNPTFSLS
AMVVNTFKLK SNVRSFNLGG MGCSAGVIAI DLAKDLLHVH KNTYALVVST
ENITNNIYAG DNRSMNVSNC LFRVGGAAIL LENKFGDRRR SKYELVHTVR
THTGADDXSF CVQQGDDBN GKIGVSLSKD IDDVAGRTVK KNIATLGFLI
LPLSKKLEFF VTFMGKHKF DKIKHYVPD FKLAIDHFCI HAGGRAVIDE
LEKNLGLSFI DVEASRSTLH RFGNTSSSSI WYELAYIEAK GRMKKGNKAW
QIALGSGFKC NSAVWVALRN VKASANSPWQ HCIDRYPVKI DSDLSKSKTH
VONGRS

FIGURE 1-7

60043919.doc

Arabidopsis thaliana FAE1 (SEO ID NO:1)

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FIGURE 2-1

Brassica napus elongase KCS (SEQ ID NO:3)

atgacgteca ttaacgtaaa geteetttae cattacgtea taaccaacet tttcaacctt tgcttctttc cgttaacggc gatcgtcgcc ggaaaagcct ateggettae catagacgat etteaceact tatactatte etateteeaa cacaacctca taaccatege tecactettt geetteaccg tttteggtte ggttctctac atcgcaacce ggcccaaacc ggtttacctc gttgagtact catgotacct tocaccaacg cattgtagat caagtatote caaggtcatg gatatetttt ateaagtaag aaaagetgat eettetegga acggeacgtg cgatgactcg tcgtggcttg acttcttgag gaagattcaa gaacgttcag gtctaggcga tgaaactcac gggcccgagg ggctgcttca ggtccctccc cggaagactt ttgcggcggc gcgtgaagag acggagcaag ttatcattgg tgcgctagaa aatctattca agaacaccaa cgttaaccct aaagatatag gtatacttgt ggtgaactca aqcatgttta atccaactcc atcgctctcc gcgatggtcg ttaacacttt caagctccga agcaacgtaa gaagctttaa ccttggtggc atgggttgta gtgccggcgt tatagccatt gatctagcaa aggaettgtt geatgteeat aaaaataegt atgetettgt ggtgageaca gagaacatca cttataacat ttacgctggt gataataggt ccatgatggt ttcaaattgc ttgttccgtg ttggtggggc cgctattttg ctctccaaca agectggaga tegtagaegg tecaagtaeg agetagttea caeggttega acqcataccq gagctgacqq caaqtctttt cqttqcqtqc aacaaqqaqa cgatgagaac ggcaaaatcg gagtgagttt gtccaaggac ataaccgatg ttgctggtcg aacggttaag aaaaacatag caacgttggg tccgttgatt cttccgttaa gcgagaaact tcttttttc gttaccttca tgggcaagaa acttttcaaa gataaaatca aacattacta cgtcccggat ttcaaacttg ctattqacca tttttgtata catgccggag gcagagccgt gattgatgtg ctagagaaga acctagccct agcaccgatc gatgtagagg catcaagatc aacgttacat agatttggaa acacttcatc tagctcaata tggtatgagt tggcatacat agaagcaaaa ggaaggatga agaaaggtaa taaagtttgg cagattqctt tagggtcagg ctttaagtgt aacagtgcag tttgggtggc totaaacaat gtoaaagott ogacaaatag toottoggaa cactgoatog acagatacco ggtcaaaatt gattotgatt caggtaagto agagactogt gtccaaaacg gtcggtccta ataa

B. napus elongase KCS (SEQ ID NO:5)

tagagogtaa oggaccacaa aagaggatoo atacaaatac atotoatogo ttccattact attctccgac acacacatg agcaatgacg tccattaacg taaageteet ttaccattac gteataacea acetttteaa eetttgttte tttccattaa cggcgatcgt cgccggaaaa gcctatctta ccatagacga tottcaccac tratactatt cotatotcca acacaacctc ataaccattg ctccactctt qqccttcacc qttttcqqtt cqqttctcta catcqcaacc eggeceaaac eggtttacet egtggagtac teatgetace ttecaceaac gcattgtaga tcaagtatct ccaaggtcat ggatatcttt ttccaagtaa gaaaagctga teettetegg aacggcacgt gegatgacte gteetggett gacttettga ggaagattea agaacgttea ggtetaggeg atgaaaccea egggeeegag gggetgette aggteeetee eeggaagaet tttgegegeg cgcgtgaaga gacggagcaa gttatcattg gtgcgctaga aaatctattc aagaacacca atgttaaccc taaagatata ggtatacttg tggtgaactc aagcatgttt aatccaactc cttcgctctc cgcgatggtc gttaacactt tcaagctccg aagcaacgta agaagcttta accttggtgg catgggttgt agtgccggcg ttatagccat tgatctagca aaggacttgt tgcatgtcca taaaaatacg tatgctcttg tggtgagcac agagaacatc acttataaca tttacgctgg tgataatagg tccatgatgg tttcaaattg cttgttccgt gttggtgggg ccgctatttt gctctccaac aagcctagag atcgtagacg gtccaagtac gagctagttc acacggttcg aacgcatacc ggagctgacg acaagtettt tegttgegtg caacaaggag acgatgagaa eggecaaace qqaqtqaqtt tqtccaaqqa cataaccqat qttqctqqtc gaacggttaa gaaaaacata gcaacgctgg gtccgttgat tcttccgtta agcgagaaac ttetttttt cottacette atgggcaaga aacttteaa agacgaaate aaacattatt acgtcccgga cttcaagctt gctatcgacc atttttgtat acatgccgga qgcaaagccg tgattgatgt gctagagaag aacctaggcc tagcaccgat cgatgtagag gcatcaagat caacgttaca tagatttaga aacacticat ctagctcaat atggtatgag ttggcataca tagaacccaa aggaaggatg aagaaaggta ataaagtttg gcagattgct ttagggtcag gctttaagtg taacagtgca gtttgggtgg ctctaaacaa tgtcaaagct tcaacaaata gtccttggga acactgcatc gacagatacc cggttaaaat tgattctgat tcaggtaagt cagagactcg tgtcccaaac ggtcggtcct aataaatgat gtttgctctc tttcgtttct ttttattggt tataataatt tgatggccac gatgtttctc ttgtttgtta tgaataaaga atcccacggt gttctagtaa aaaaaaaaaa aaaaaaaaa aaaaaa

FIGURE 2-3

PCT/US01/18737

WO 01/94565

At114 (SEQ ID NO:7)

atgacgtccg ttaacgttaa gctcctttac cgttatgtct taaccaactt tttcaacctc tgtttgttcc cgttaacggc gttcctcgcc ggaaaagcct ctcggcttac cataaacgat ctccacaact tcctttccta tctccaacac aaccttataa cagtaacttt actctttgct ttcactgttt tcggtttggt tototacato gtaaccogac ccaatcoggt ttatctcgtt gactactcgt gttaccttcc qccaccqcat ctcaaaqtta gtgtctctaa agtcatggat attttctacc aaataagaaa agctgatact tcttcacgga acggcacgtg tgatgattcg tcgtggcttg acttcttgag gaagattcaa gaacgttcag gtctaggcga tgaaactcac gggcccgagg ggctgcttca ggtccctccc cggaagactt ttgcggcggc gcgtgaagag acggagcaag ttatcattgg tgcgctagaa aatctattca agaacaccaa cgttaaccct aaagatatag gtatacttgt ggtgaactca agcatgttta atccaactcc atcgctctcc gcgatggtcg ttaacacttt caagctccga agcaacgtaa gaagctttaa ccttggtggc atgggttgta gtgccggcgt tatagccatt gatctagcaa aggacttgtt gcatgtccat aaaaatacgt atgctcttgt ggtgagcaca gagaacatca cttataacat ttacgctggt gataataggt ccatgatggt ttcaaattgc ttgttccgtg ttggtggggc cgctattttg ctctccaaca agcetggaga tegtagaegg tecaagtaeg agetagttea eaeggttega acqcataccg gagctgacgg caagtctttt cgttgcgtgc aacaaggaga cgatgagaac ggcaaaatcg gagtgagttt gtccaaggac ataaccgatg ttgctggtcg aacggttaag aaaaacatag caacgttggg tccgttgatt cttccgttaa gcgagaaact tctttttttc gttaccttca tgggcaagaa acttttcaaa gataaaatca aacattacta cgtcccggat ttcaaacttg ctattgacca tttttgtata catgccggag gcagagccgt gattgatgtg ctagagaaga acctagccct agcaccgatc gatgtagagg catcaagatc aacgttacat agatttggaa acacttcatc tagctcaata tggtatgagt tggcatacat agaagcaaaa ggaaggatga agaaaggtaa taaagtttgg cagattgctt tagggtcagg ctttaagtgt aacagtgcag tttgggtggc totaaacaat gtcaaagctt cgacaaatag toottgggaa cactgcatcg acagataccc ggtcaaaatt gattctgatt caggtaagtc agagactcgt gtcccaaacg gtcggtccta a

At74 (SEO ID NO:9)

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At114 L91C K92R (SEO ID NO:11)

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At114 K92R (SEO TD NO:13)

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At114 G307D (SEO ID NO:15)

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At74 G306D (SEO ID NO:17)

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At114 L91C K92R G307D (SEO ID NO:19)

atgacgtccg ttaacgttaa gctcctttac cgttatgtct taaccaactt tttcaacctc tgtttgttcc cgttaacggc gttcctcgcc ggaaaagcct ctcggcttac cataaacgat ctccacaact tcctttccta tctccaacac aaccttataa cagtaacttt actctttgct ttcactgttt tcggtttggt tetetacate gtaacecgac ccaatecggt ttatetegtt gactactegt gttaccttcc gccaccgcat tgcagagtta gtgtctctaa agtcatggat attttctacc aaataagaaa agctgatact tcttcacgga acggcacgtg tgataattcg tcgtggcttg acttcttgag gaagattcaa gaacqttcag gtctaggega tgaaactcac gggcccgagg ggctgcttca ggtccctccc cggaagactt ttgcggcggc gcgtgaagag acggagcaag ttatcattgg tgcgctagaa aatctattca agaacaccaa cgttaaccct aaagatatag gtatacttgt ggtgaactca agcatgttta atccaactcc atcgctctcc qcqatqqtcq ttaacacttt caagctccqa agcaacgtaa qaagctttaa ccttggtggc atgggttgta gtgccggcgt tatagccatt gatctagcaa aggacttgtt gcatgtccat aaaaatacgt atgctcttgt ggtgagcaca gagaacatca cttataacat ttacgctggt gataataggt ccatgatggt ttcaaattgc ttgttccgtg ttggtggggc cgctattttg ctctccaaca agcetggaga tegtagaegg tecaagtaeg agetagttea caeggttega acgcataccg gagctgacga caagtctttt cgttgcgtqc aacaaggaga cqatgagaac ggcaaaatcg gagtgagttt gtccaaggac ataaccgatg ttgctggtcg aacggttaag aaaaacatag caacgttggg tccgttgatt cttccqttaa qcqaqaaact tcttttttc qttaccttca tqqqcaaqaa acttttcaaa gataaaatca aacattacta cgtcccggat ttcaaacttg ctattgacca tttttgtata catgccggag gcagagccgt gattgatgtg ctagagaaga acctagccct agcaccgatc gatgtagagg catcaagatc aacqttacat agatttggaa acacttcatc tagctcaata tggtatgagt tggcatacat agaagcaaaa ggaaggatga agaaaggtaa taaagtttgg cagattgctt tagggtcagg ctttaagtgt aacagtgcag tttgggtggc totaaacaat gtcaaagctt cgacaaatag toottgggaa cactgcatcg acagataccc ggtcaaaatt gattctgatt caggtaagtc agagactcgt otccaaaaco otcootccta a

FIGURE 2-10

At114 K92R G307D (SEQ ID NO:21)

atgacgtccg ttaacgttaa gctcctttac cgttatgtct taaccaactt tttcaacete tgtttgttcc cgttaacggc gttcctcgcc ggaaaagcct ctcggcttac cataaacgat ctccacaact tcctttccta tctccaacac aaccttataa cagtaacttt actctttgct ttcactgttt tcggtttggt tototacato gtaaccogac ccaatcoggt ttatotogtt gactactogt gttacettee gecacegeat etcagagtta gtgtetetaa agteatggat attttctacc aaataagaaa agctgatact tcttcacgga acggcacgtg tgatgattcg tcgtggcttg acttcttgag gaagattcaa gaacgttcag gtctaggcga tgaaactcac gggcccgagg ggctgcttca ggtccctccc cggaagactt ttgcggcggc gcgtgaagag acggagcaag ttatcattgg tgcgctagaa aatctattca agaacaccaa cgttaaccct aaagatatag gtatacttqt qqtqaactca aqcatqttta atccaactcc atcqctctcc gcgatggtcg ttaacacttt caagctccga agcaacgtaa gaagctttaa ccttggtggc atgggttgta gtgccggcgt tatagccatt gatctagcaa aggacttgtt gcatgtccat aaaaatacgt atgctcttgt ggtgagcaca gagaacatca cttataacat ttacgctggt gataataggt ccatgatggt ttcaaattqc ttqttccqtq ttqqtqqqqc cqctattttq ctctccaaca agcotggaga togtagacgg tocaagtacg agctagttca cacggttcga acgcataccg gagctgacga caagtctttt cgttgcgtgc aacaaggaga cgatgagaac ggcaaaatcg gagtgagttt gtccaaggac ataaccgatg ttgctggtcg aacggttaag aaaaacatag caacgttggg tccgttgatt cttccgttaa gcgagaaact tcttttttc gttaccttca tgggcaagaa actiticaaa gataaaatca aacattacta cgtcccggat ticaaacttg ctattgacca tttttgtata catgccggag gcagagccgt gattgatgtg ctaqaqaaqa acctagccct agcaccgatc gatgtagagg catcaagatc aacgttacat agatttggaa acacttcatc tagctcaata tggtatgagt tggcatacat agaagcaaaa ggaaggatga agaaaggtaa taaagtttgg cagattqctt tagggtcagg ctttaagtgt aacagtqcag tttgggtggc totaaacaat gtoaaagott ogacaaatag toottgggaa cactgoatog acagataccc ggtcaaaatt gattctgatt caggtaagtc agagactcgt gtccaaaacg gtcggtccta a

At254 (SEQ ID NO:23)

atgacgtccg ttaacgttaa gctcctttac cgttacgtct taaccaactt tttcaacctc tgtttgttcc cgttaacggc gttcctcgcc ggaaaagcct ctcggcttac cataaacgat ctccacaacc tcctttccta tctccaacac aaccttataa cagtaacttt actctttgct ttcactgttt tcggtttggt tetetacate gtaaccegae ecaateeggt ttatetegtt gactactegt gttaccttcc accaccqcat ctcaaaqtta qtqtctctaa aqtcatqqat attttctacc aaataagaaa agctgatact tcttcacgga acgtggcatg tgatgatccg tectegeteg atttectgag gaagatteaa gagegtteag gtctaggtga tgagacgtac agtcctgagg gactcattca cgtaccaccg cqqaaqactt ttqcaqcqtc acqtqaaqaq acaqaqaaqg ttatcatcqq tgcgctcgaa aatctattcg agaacaccaa agttaaccct agagagattg gtatacttgt ggtgaactca agcatgttta atccaactcc ttcgctatcc gctatggtcg ttaatacttt caagctccga agcaacatca aaagctttaa tctaggagga atgggttgta gtgctggtgt tattgccatt gatttggcta aagacttgtt gcatgttcat aaaaacactt atgctctcgt ggtgagcaca gagaacatca cttataacat ttacgctggt gataataggt ccatgatggt ttcaaattgc ttgttccgtg ttggtggggc cgctattttg ctctccaaca agcctggaga tcgtagacgg tccaagtacg agctagttca cacggttcga acgcataccg gagctgacgg caagtctttt cgttgcgtgc aacaaggaga cgatgagaac ggcaaaatcg gagtgagttt gtccaaggac ataaccgatg ttgctggtcg aacggttaag aaaaacatag caacgttggg tccgttgatt cttccqttaa qcqaqaaact tcttttttc gttaccttca tqqqcaagaa acttttcaaa gataaaatca aacattacta cgtcccggat ttcaaacttg ctattgacca tttttgtata catgccggag gcagagccgt gattgatgtg ctagagaaga acctagccct agcaccgatc gatgtagagg catcaagatc aacgttacat agatttggaa acacttcatc tagctcaata tggtatgagt tggcatacat agaagcaaaa ggaaggatga agaaaggtaa taaagtttgg cagattoctt tagggtcagg ctttaagtgt aacagtgcag tttgggtggc totaaacaat gtoaaagott cgacaaatag toottgggaa cactgcatog acagataccc ggtcaaaatt gattctgatt caggtaagtc agagactcgt gtcccaaacg gtcggtccta a

At173 (SEO ID NO:25)

atgacgtccg ttaacgttaa gctcctttac cgttacgtct taaccaactt tttcaacctc tgtttgttcc cgttaacggc gttcctcgcc ggaaaagcct ctcgcttac cataaacgat ctccacaact tcctttccta tctccaacac aaccttataa cagtaacttt actctttgct ttcactgttt tcggtttggt tototacato gtaaccogac ccaatcoggt ttatotogtt gactactogt gttaccttcc accaccgcat ctcaaagtta gtgtctctaa agtcatggat attttctacc aaataagaaa agctgatact tcttcacgga acgtggcatg tgatgatecg tectegeteg attteetgag gaagatteaa gagegtteag gtctaggtga tgagacgtac agtcctgagg gactcattca cgtaccaccg eggaagaett ttgeagegte acgtgaagag acagagaagg ttateategg tgcgctcgaa aatctattca agaacaccaa cgttaaccct aaagatatag gtatacttgt ggtgaactca agcatgttta atccaactcc atcgctctcc gcgatggtcg ttaacacttt caagctccga agcaacgtaa gaagctttaa ccttggtggc atgggttgta gtgccggcgt tatagccatt gatctagcaa aggacttgtt gcatgtccat aaaaatacgt atgctcttgt ggtgagcaca gagaacatca cttataacat ttacgctggt gataataggt ccatgatggt ttcaaattgc ttgttccgtg ttggtggggc cgctattttg ctctccaaca agcctggaga togtagacgg tocaagtacg agctagttca cacggttcga acgcataccg gagctgacgg caagtctttt cgttgcgtgc aacaaggaga cgatgagaac ggcaaaatcg gagtgagttt gtccaaggac ataaccgatg ttgctggtcg aacggttaag aaaaacatag caacgttggg tccgttgatt cttccqttaa qcqaqaaact tcttttttc qttaccttca tqqqcaaqaa acttttcaaa gataaaatca aacattacta cgtcccggat ttcaaacttg ctattgacca tttttgtata catgccggag gcagagccgt gattgatgtg ctagagaaga acctagccct agcaccgatc gatgtagagg catcaagatc aacgttacat agatttggaa acacttcatc tagctcaata tggtatgagt tggcatacat agaagcaaaa ggaaggatga agaaaggtaa taaagtttgg cagattgctt tagggtcagg ctttaagtgt aacagtgcag tttgggtggc tctaaacaat gtcaaagctt cgacaaatag tccttgggaa cactgcatcg acagataccc ggtcaaaatt gattctgatt caggtaagtc agagactcgt gtecaaaacg gteggteeta a

WO 01/94565

PCT/US01/18737

Bn176 (SEQ ID NO:27)

atgacgtcca ttaacgtaaa gctcctttac cattacgtca taaccaacct tttcaacctt tgcttctttc cgttaacggc gatcgtcgcc ggaaaagcct atoggottac catagacgat ottoaccact tatactatto etatotecaa cacaacetca taaceatege tecactettt geetteaceg tttteggtte ggttctctac atcgcaaccc ggcccaaacc ggtttacctc gttgagtact catgotacct tocaccaacq cattgtagat caagtatoto caaggtcatg gatatetttt ateaagtaag aaaagetgat eettetegga acggeacgto cgatgactcg tcgtggcttg acttcttgag gaagattcaa gaacgttcag gtctaggcga tgaaactcac gggcccgagg ggctgcttca ggtccctccc cggaagactt ttgcggcggc gcgtgaagag acggagcaag ttatcattgg tgcgctagaa aatctattca agaacaccaa agttaaccct agagagattg gtatacttqt qqtqaactca agcatqttta atccaactcc ttcqctatcc gctatggtcg ttaatacttt caagctccga agcaacatca aaagctttaa tctaggagga atgggttgta gtgctggtgt tattgccatt gatttggcta aagacttgtt gcatgttcat aaaaacactt atgctcttgt ggtgagcact gagaacatca cacaaggcat ttatgctgga gaaaatagat caatgatggt tageaattgc ttgtttcgtg ttggtggggc cgcgattttg ctctctaaca agtegggaga ceggagaegg tecaagtaca agetagttea caeggteega acgcatactg gagctgatga caagtctttt cgatgtgtgc aacaagaaga tgatgagage ggcaaaatcg gagtttgtct gtcaaaggac ataaccaatg ttgcggggac aacacttacg aaaaatatag caacattggg tccgttgatt cttcctttaa gcgaaaagtt tctttttttc gctaccttcg tcgccaagaa acttctaaag gataaaatca agcattacta tgttccggat ttcaagcttg ctgttgacca tttctgtatt catgccggag gcagagccgt gatcgatgag ctagagaaga acttaggact atcgccgatc gatgtggagg catctagatc aacgttacat agatttggga atacttcatc tagctcaatt tggtatgaat tagcatacat agaggcaaag ggaagaatga agaaagggaa taaagcttgg cagattgctt taggatcagg gtttaagtgt aatagtgcgg tttgggtggc totacgcaat gtcaaggcat cggcaaatag toottggcaa cattgcatcg atagatatcc ggttaaaatt gattctgatt tgtcaaagtc aaagactcat gtccaaaacg gtcggtccta a

At399 (SEQ ID NO:29)

atgacgtccg ttaacgttaa gctcctttac cgttacgtct taaccaactt tttcaacctc tgtttgttcc cgttaacggc gttcctcgcc ggaaaagcct ctcggcttac cataaacgat ctccacaact tcctttccta tctccaacac aaccttataa cagtaacttt actctttgct ttcactgttt tcggtttggt tetetacate gtaaccegae ccaatceggt ttatetegtt gactactegt gttaccttcc accaccgcat ctcaaagtta gtgtctctaa agtcatggat attttctacc aaataagaaa agctgatact tcttcacgga acgtggcatg tgatgatccg tectegetcg atttectgag gaagatteaa gagegtteag qtctaqqtga tgagacgtac agtcctgagg gactcattca cgtaccaccg cggaagactt ttgcagcgtc acgtgaagag acagagaagg ttatcatcgg tgcgctcgaa aatctattcg agaacaccaa agttaaccct agagagattg gtatacttgt ggtgaactca agcatgttta atccaactcc ttcgctatcc gctatggtcg ttaatacttt caagctccga agcaacatca aaagctttaa tctaggagga atgggttgta gtgctggtgt tattgccatt gatttggcta aagacttgtt gcatgttcat aaaaacactt atgctcttgt ggtgagcact gagaacatca cacaaggcat ttatgctgga gaaaatagat caatgatggt tagcaattgc ttgtttcgtg ttggtggggc cgcgattttg ctctctaaca agtogggaga coggagacgg tocaagtaca agctagttca cacggtocga acgcatactg gagctgatga caagtctttt cgatgtgtgc aacaagaaga cgatgagage ggcaaaatcg gagtttgtct gtcaaaggac ataaccaatg ttgcggggac aacacttacg aaaaatatag caacattggg tccgttgatt cttcctttaa gcgaaaagtt tctttttttc gctaccttcg tcgccaagaa acttctaaag gataaaatca agcattacta tgttccggat ttcaagcttg ctgttgacca tttctgtatt catgccggag gcagagccgt gatcgatgtg ctagagaaga acctagecet ageacegate gatgtagagg cateaagate aacqttacat agatttggaa acacttcatc tagctcaata tggtatgagt tggcatacat agaagcaaaa ggaaggatga agaaaggtaa taaagtttgg cagattgett tagggtcagg ctttaagtgt aacagtgcag tttgggtggc tctaaacaat gtcaaagctt cgacaaatag tccttgggaa cactgcatcg acagataccc gqtcaaaatt gattctgatt caggtaagtc agagactcqt gtccaaaacg gtcggtccta a

Bn399 (SEO ID NO:31)

atgacqtcca ttaacqttaa qctcctttac cattacqtca taaccaacct tttcaacctt tgcttctttc cgttaacggc gatcgtcgcc ggaaaagcct atcggcttac catagacgat cttcaccact tatactattc ctatctccaa cacaacetca taaccatege tecactettt geetteaceg tttteggtte ggttctctac atcgcaaccc ggcccaaacc ggtttacctc gttgagtact catgetacet tecaceaacg cattgtagat caagtatete caaggteatg gatatettt atcaagtaag aaaagetgat cettetegga acggcacgtg cgatgactcg tcgtggcttg acttcttgag gaagattcaa gaacgttcag gtctaggcga tgaaactcac gggcccgagg ggctgcttca ggtccctccc cggaagactt ttgcggcggc gcgtgaagag acggagcaag ttatcattgg tgcgctagaa aatctattca agaacaccaa cgttaaccct aaagatatag gtatacttgt ggtgaactca aqcatgttta atccaactcc atcgctctcc gcgatggtcg ttaacacttt caagctccga agcaacgtaa gaagctttaa cettggtgge atgggttgta gtgccggcgt tatagccatt gatctagcaa aggacttgtt gcatgtccat aaaaatacgt atgctcttgt ggtgagcaca gagaacatca cttataacat ttacgctggt gataataggt ccatgatggt ttcaaattgc ttgttccgtg ttggtggggc cgctattttg ctctccaaca agcctggaga tcgtagacgg tccaagtacg agctagttca cacggttcga acqcataccq gagctgacgg caagtetttt cgttgcgtgc aacaaggaga cgatgagaac ggcaaaatcg gagtgagttt gtccaaggac ataaccgatg ttgctggtcg aacggttaag aaaaacatag caacgttggg tccgttgatt cttccqttaa qcqaqaaact tcttttttc gttaccttca tgggcaagaa acttttcaaa gataaaatca aacattacta cgtcccggat ttcaaacttg ctattgacca tttttgtata catgccggag gcagagccgt gatcgatgag ctagagaaga acttaggact atcgccgatc gatgtggagg catctagatc aacgttacat agatttggga atacttcatc tagctcaatt tggtatgaat tagcatacat agaggcaaag ggaagaatga agaaagggaa taaagcttgg cagattgctt taggatcagg gtttaagtgt aatagtgcgg tttgggtggc tctacgcaat gtcaaggcat cggcaaatag tccttggcaa cattgcatcg atagatatcc gqttaaaatt gattctqatt tqtcaaaqtc aaaqactcat gtccaaaacg gtcggtccta a

Bn G307D (SEO ID NO:33)

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At K92R (SEO ID NO:35)

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